ผลของการลดระดับโปรตีนในอาหาร และใช้มันสำปะหลังทดแทนข้าวโพดในอาหารไก่เนื้อต่อสมรรถภาพการเจริญเติบโต การย่อยได้ของโปรตีนที่ลำไส้เล็กส่วนปลาย และการขับออกของไนโตรเจนในมูล

นายนิรันดร์ บุญสินธุ์ชัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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EFFECTS OF PROTEIN REDUCTION AND SUBSTITUTION OF CASSAVA FOR CORN IN BROILER DIET ON GROWTH PERFORMANCE, ILEAL PROTEIN DIGESTIBILITY AND NITROGEN EXCRETION IN FECES

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นิรันดร์ บุญสินธุ์ชัย : ผลของการลดระดับโปรตีนในอาหาร และใช้มันสำปะหลังทดแทนข้าวโพดในอาหารไก่เนื้อ ต่อสมรรถภาพการเจริญเติบโต การย่อยได้ของโปรตีนที่ลำไส้เล็กส่วนปลาย และการขับออกของไนโตรเจนในมูล (EFFECTS OF PROTEIN REDUCTION AND SUBSTITUTION OF CASSAVA FOR CORN IN BROILER DIET ON GROWTH PERFORMANCE, ILEAL PROTEIN DIGESTIBILITY AND NITROGEN EXCRETION IN FECES) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : รศ. สุวรรณา กิจภากรณ์, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : ดร. มานพ พจนากรณ์, 53 หน้า.

การทดลองครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของการลดระดับโปรตีน และการทดแทนข้าวโพดด้วยมันสำปะหลัง ต่อประสิทธิภาพการเจริญเติบโต การย่อยได้ของโปรตีนในลำไส้เล็กส่วนปลาย และการขับออกของไนโตรเจนในมูลของไก่เนื้อ โดยใช้ไก่เนื้อเพศผู้สายพันธุ์ Cobb 500 อายุ 1 วัน จำนวน 2,688 ตัว สุ่มแบ่งออกเป็น 8 กลุ่มๆ ละ 6 ซ้ำๆ ละ 56 ตัว แผนการทดลองเป็นแบบ 2 X 4 แฟคตอเรียล อาหารทดลองประกอบด้วยอาหารที่มีระดับโปรตีนแตกต่างกัน 4 ระดับ คือ 100, 95, 90 และ 85% ของระดับสารอาหารที่ผู้ผลิตสายพันธุ์แนะนำในช่วง 1-21 และ 22-42 วัน และใช้แหล่งพลังงานจากข้าวโพด และจากข้าวโพดที่ทดแทนด้วยมันสำปะหลัง 50% อย่างละ 4 สูตร รวมทั้งหมด 8 สูตร โดยคงระดับกรดอะมิโนที่จำเป็น 4 ชนิดได้แก่ เมทไธโอนีน ไลซีน ทรีโอนีน และทริปโตแฟน ตามคำแนะนำในแต่ละช่วงอายุ ผลการทดลองในช่วงอายุ 1-21 วันไม่พบผลจากแหล่งพลังงาน และอิทธิพลร่วมของระดับโปรตีนและแหล่งพลังงาน แต่พบผลของความแตกต่างของระดับโปรตีนต่ออัตราการแลกเนื้อ โดยที่กลุ่มที่ได้รับอาหารโปรตีน 85% มีค่าสูงกว่ากลุ่มอื่น($P<0.05$) และการขับออกของไนโตรเจนในมูลที่ลดลงตามระดับโปรตีนในอาหารที่ลดต่ำลง($P<0.05$) แต่ไม่มีผลต่อการย่อยได้ของโปรตีน ($P>0.05$) ในช่วงอายุ 22-42 วันพบความแตกต่างของอิทธิพลร่วมระหว่างระดับโปรตีนและแหล่งพลังงานต่อปริมาณอาหารที่กิน โดยไก่ที่ได้รับข้าวโพดร่วมกับมันสำปะหลัง ให้อัตราการแลกเนื้อสูงกว่าไก่ที่ได้รับข้าวโพดเป็นแหล่งพลังงาน ($P<0.001$) และพบความแตกต่างของระดับโปรตีน โดยไก่กลุ่มที่ได้รับปริมาณโปรตีนต่ำสุดมีค่าการย่อยได้ของโปรตีนและไนโตรเจนที่ขับออกในมูลต่ำกว่ากลุ่มอื่น ($P<0.05$) ขณะที่แหล่งพลังงานพบว่าไก่ที่กินอาหารที่มีข้าวโพดเป็นแหล่งพลังงานมีการย่อยได้ของโปรตีนดีกว่ากลุ่มที่ใช้มันสำปะหลังทดแทนข้าวโพด 50% ($P<0.0001$) สรุปว่าสามารถลดระดับโปรตีนลงเหลือ 95% และสามารถใช้มันสำปะหลังทดแทนข้าวโพดได้ 50% เมื่อพิจารณาเฉพาะสมรรถภาพการเจริญเติบโตและผลตอบแทนทางเศรษฐกิจ อย่างไรก็ตามเมื่อนำผลกระทบต่อสิ่งแวดล้อมเข้ามาพิจารณาร่วมด้วย ระดับโปรตีนสามารถลดลงเหลือ 90% ของระดับสารอาหารที่ผู้ผลิตสายพันธุ์แนะนำ

ภาควิชา สัตวบาล…… ลายมือชื่อนิสิต……

สาขาวิชา อาหารสัตว์…… ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก……

ปีการศึกษา 2553…… ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม……

5175578331 MAJOR ANIMAL NUTRITION
KEYWORDS: PROTEIN REDUCTION / CASSAVA / CORN / GROWHT PERFORMANCE / ILEAL PROTEIN DIGESTIBILITY / FECAL NITROGEN EXCRETION / BROILER

NIRUN BOONSINCHAI: EFFECTS OF PROTEIN REDUCTION AND SUBSTITUTION OF CASSAVA FOR CORN IN BROILER DIET ON GROWTH PERFORMANCE, ILEAL PROTEIN DIGESTIBILITY AND NITROGEN EXCRETION IN FECES. ADVISOR : ASSOC. PROF. SUWANNA KIJPARKORN. CO-ADVISOR : DR. MANOP POTCHANAKORN, 53 pp.

This experiment was conducted to investigate the effect of protein reduction and substitution of cassava for corn on growth performance, ileal protein digestibility and nitrogen excretion in the feces of broilers. Total 2,688 day old male (Cobb 500) broilers were randomly divided into 8 groups with 6 replicates of 56 birds each. The 2 x 4 factorial arrangements were applied to perform 8 diets with 4 protein levels (100%, 95%, 90%, and 85% of primary breeder recommendation during 1-21 and 22-42 days of age) and 2 energy sources (Corn; CO and Corn substituted with Cassava 50%; CC) while maintaining a constant level of 4 essential amino acids (Met, Lys, Thr, and Trp) to meet the recommendation in each period. At 1-21 days of age, there were no effects from energy sources and interaction between protein levels and energy sources on growth performance. Protein levels affected on both FCR and nitrogen excretion but did not influence ileal protein digestibility. The 85% protein group had significantly higher FCR ($P<0.05$) than other protein groups, while fecal nitrogen decreased as dietary protein levels declined ($P<0.05$). At 22-42 days of age, there was interaction between energy sources and protein levels on feed intake. Birds in CC group had significantly higher FCR than CO group ($P<0.001$) and also found the difference in protein levels. Broilers fed the lowest protein level diet had significantly lower ileal protein digestibility and nitrogen excretion than other protein groups ($P<0.05$). Birds fed CO diets had significantly higher protein digestibility than CC diets ($P<0.0001$). In conclusion, dietary crude protein can be reduced up to 95% of recommendation and cassava can be used to substitute for corn by 50% when considered only growth performance and economic returns. However, when environmental pollution was included, the crude protein could be decreased to 90% of recommendation.

Department : Animal Husbandry……..
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Student's Signature……
Advisor's Signature……
Co-advisor's Signature……..

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LIST OF ABBREVIATION

ADG	=	Average daily gain
Arg	=	Arginine
Ala	=	Alanine
ANOVA	=	Analysis of variance
AOAC	=	Association of Official Analytical Chemists
Asn	=	Asparagine
Asp	=	Aspartic acid
CC	=	Corn & cassava
CO	=	Corn
CP	=	Crude protein
Cys	=	Cystine
DM	=	Dry matter
EAA	=	Essential amino acid
EEF	=	European Efficiency Factor
FCR	=	Feed conversion rate
FFSB	=	Full fat soybeans
GE	=	Gross energy
GI tract	=	Gastrointestinal tract
Gln	=	Glutamine
Glu	=	Glutamic acid
Gly	=	Glycine
His	=	Histidine
Hyl	=	Hydroxylysine
Hyp	=	Hydroxyproline
Ile	=	Isoleucine
Leu	=	Leucine
LSD	=	Least significant difference
Lys	=	Lysine

ME	=	Metabolizable energy
Met	=	Methionine
NEAA	=	Non-essential amino acid
NRC	=	National Research Council
NSP	=	Non-starch polysaccharide
Phe	=	Phenylalanine
Pro	=	Proline
SBM	=	Soybean meal
SDS	=	Slowly digestible starch
Ser	=	Serine
SI	=	Small intestine
SID	=	Standardized ileal digestibility
Thr	=	Threonine
Trp	=	Tryptophan
Tyr	=	Tyrosine
TSAA	=	Total sulfur amino acid
Val	=	Valine

CHAPTER I
INTRODUCTION AND AIMS

Environmental pollution is a big issue around the world. Pollution is produced from both industrial and agricultural sectors, for instance poultry production. Nitrogen excreted with poultry feces can also contaminate into natural water sources and the atmosphere (Nahm, 2007). The exceeding threshold levels of both reduced and oxidized forms of nitrogen can cause many problems according to Ndegwa et al. (2008); respiratory diseases, algae blooms, low water quality, imbalance ecosystem, climatic changes, and soil acidification. Therefore, reduction of nitrogen in the broiler feces is one of the methods to help reduce those problems.

The level of nutrients used for maintenance, growth, and production in broiler feeds should be considered. Broiler cannot digest and absorb all of nutrients, especially when nutrients are imbalanced. When partial amino acids exceeding the requirement of animals are broken down, carbon is used for energy whereas nitrogen is excreted in feces in the case of poultry (Todd and Angel, 2008). There are several methods to reduce nitrogen excretion in feces include: formulating the balanced diet, using high digestibility ingredients, supplementing enzyme, improving feed processing, using the phase feeding, and also reducing protein levels with adding synthetic essential amino acids (Nahm, 2007). Ferket et al. (2002) mentioned that the most appropriate way to reduce nitrogen excretion in feces is decreasing protein levels in the diet with supplementation of synthetic amino acids. Reducing 3 percentages unit of protein levels from requirement in each dietary period at the constant energy level didn't show any negative effect on growth performance and carcass traits (Oviedo-Rondon et al., 2005). While Zhao et al. (2009) reported that reducing 4% protein in the diets with adding synthetic amino acids and decreased energy to maintain ME to protein ratio has negative effect on growth performance. Nevertheless, previous researches showed that constant energy and reducing protein levels without maintaining essential amino acid levels to meet or above the minimum level of NRC (1994) recommendation gave

negative effect on growth performance (Alertor et al., 2000; Kamran et al., 2004; Corzo et al., 2005).

Maintaining essential amino acids in low protein diet can be accomplished by supplementation of marketable synthetic amino acids such as methionine (Met), lysine (Lys), threonine (Thr), and tryptophan (Trp). Those amino acids are considered as the first to fourth essential amino acids in poultry, respectively (Ojano-Dirain and Waldroup, 2002; Rosa et al., 2001). Therefore, decreasing protein levels in the diets with supplementation of these amino acids to meet the requirement in each period of broiler age according to NRC (1994) or the recommendation of breeder companies may help to reduce nitrogen excretion in feces.

Energy is one of the important factors for utilizing amino acids. If energy is insufficient, some amino acids will be broken down to produce energy which was mentioned before. From the review literatures, most studies used corn as an energy source but nowadays corn price is very high. Thailand had high potential to produce cassava which has lower price than corn and the previous study by Saentaweesuk et al. (2000) indicated that the highest replacement level of cassava for corn without any effect on growth performance was 50%.

The objectives of the present study were to determine the effect of reducing protein levels by maintaining 4 essential amino acids according to the primary breeder recommendation and using 2 energy sources (corn and substitution of cassava for corn 50%) on growth performance, ileal protein digestibility, economic returns and nitrogen excretion in feces of broilers.

CHAPTER II
BACKGROUND INFORMATION

2.1 Protein and amino acids function in animal

Protein is a chain of amino acids connected together by peptide bond. Crude protein is commonly used to describe the protein content of diets (Fuller, 2004). Protein is the important parts of the soft tissues in the body such as muscle, connective tissue, collagen, skin, feathers, toenails and the horny portion of the beak. There are several functions of blood protein, for example; albumin and globulins help to maintain homeostasis, regulate osmotic pressure, and act as a reserve supply of amino acids. Fibrinogen and thromboplastin involve in blood clotting. Hemoglobin carries oxygen to the cells. Lipoproteins transport fat-soluble vitamins and other fatty metabolites. (Leeson and Summers, 2001). In addition, protein is crucial for maintenance, growth, and production of animals. It also is a component of enzymes and hormones (Perry et al., 1999).

Amino acids are organic acids composed of carbon (C), hydrogen (H), oxygen (O), nitrogen (N), sulfur (S), and selenium (Se). They are the basic units to form protein and several products that are required for animal function (Fuller, 2004).

2.2 Classification of amino acids

Unlike plants, animals can't synthesize all of the amino acids. Amino acids which can't be synthesized by animals and therefore must be supplied in the diet are classified as the essential or indispensable amino acids. While amino acids that can be synthesized by the animal are termed non-essential or dispensable amino acids. However, some of them can't be synthesized at an enough rate for maximum growth and therefore should be also supplied in the diet are called semi-essential amino acids. The essential and non-essential amino acids involved in protein synthesis are shown in Table 2.1

Table 2.1 Nutritional classification of amino acids

Essential amino acids	Semi-essential amino acids*	Non-essential amino acids
Arginine (Arg)	Tyrosine (Tyr)	Alanine (Ala)
Lysine (Lys)	Cystine (Cys)	Aspartic acid (Asp)
Histidine (His)	Hydroxylysine (Hyl)	Asparagine (Asn)
Leucine (Leu)		Glutamic acid (Glu)
Isoleucine (Ile)		Glutamine (Gln)
Valine (Val)		Hydroxyproline (Hyp)
Methionine (Met)		Glycine (Gly)**
Threonine (Thr)		Serine (Ser)**
Tryptophan (Trp)		Proline (Pro)***
Phenylalanine (Phe)		

*Tyrosine is synthesized form phenylalanine; cystine form methionine; hydroxylysine form lysine.

**Under some conditions glycine or serine synthesis may not be sufficient for very rapid growth; either serine or glycine may need to be supplied in the diet.

***When diets composed of crystalline amino acids are used, proline may be necessary to achieve maximum growth.

Adapted from Leeson and Summers (2001)

The essential amino acids can be classified into three categories, depend on the bird's ability to achieve limited synthesis or not. 1) Lys and Thr have no intermediary precursors, so 100% of requirement must be supplied by diet. 2) Leu, Ile and Val can be synthesized from intermediate precursor but the production is very limited, so should be supplied 2-5% of demand. 3) Arg and His can also be synthesized from intermediates during general metabolism but under unusual conditions should be add 5-8% of requirement (Leeson and Summers, 2001).

The semi-essential amino acids (Figure 2.1) can be synthesized from another essential amino acid. Cys and Tyr can be irreversibly synthesized from Met and Phe, respectively (D'Mello, 2003). Met is converted into adenosyl Met, then homocysteine and finally Cys, respectively. Tyr can be synthesized directly from hydroxylation of Phe and Hyl can be synthesized from Lys (Leeson and Summers, 2001).

Non-essential amino acids can be synthesized by transamination or other more complex reactions of metabolites produced from the oxidation of glucose or Arg from the urea cycle in mammals (Fig 2.1).Gly is synthesized from Ser or choline. The major non-essential amino acids found in tissue and feedstuffs are Gly, Ser, Ala, Glu, and Asp (Leeson and Summers, 2001).

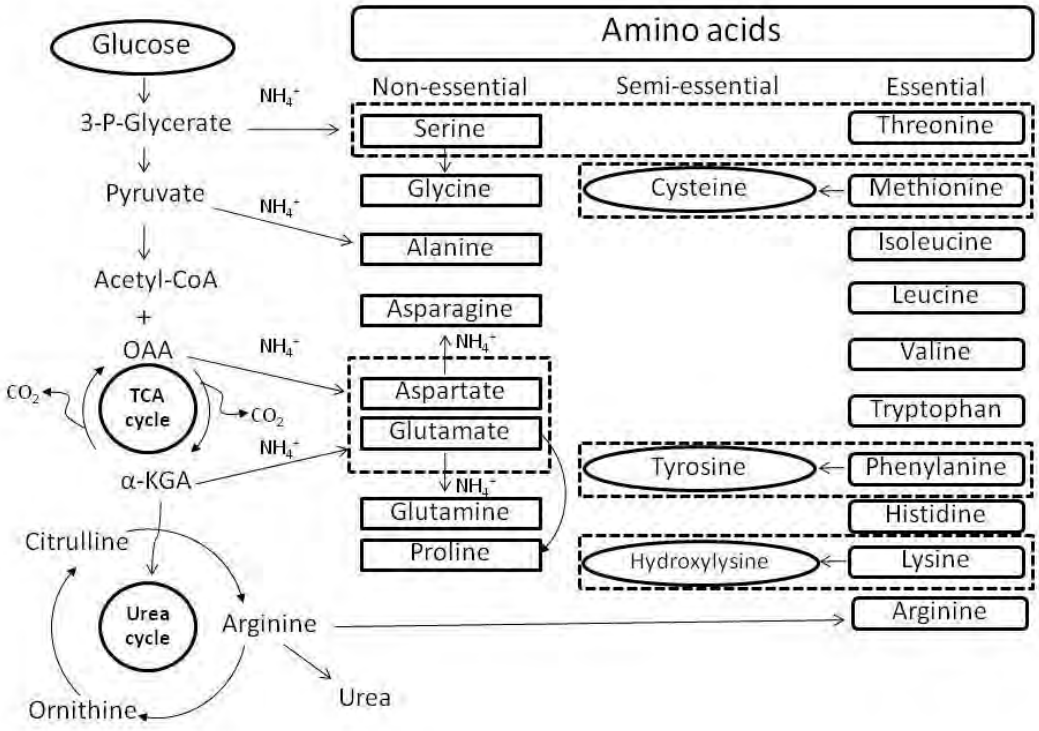


Figure 2.1 Synthesis routes of semi-essential and non-essential amino acids (Adapted from D'Mello, 2003).

2.3 Essential amino acids in broiler

2.3.1. Methionine and Cystine requirement

Methionine and/or total sulfur amino acid (TSAA) is the first limiting amino acid in broiler diets with the plenty functions in the body. Met serves as a vital portion of body protein, a precursor for Cys, and source of sulfur in the diets (Ojano-Dirain and Waldroup, 2002). Methionine serves as methyl donor for creatine, choline, and carnitine (Leeson and Summers, 2001). Met plays a major component in protein synthesis, N-

formylmethionine is also the amino acid that corresponds with most start codon (AUG) in the translation of mRNA to protein (Kim et al., 2006).

Aftab and Ashraf (2009) evaluated the requirement of Met and Cys in Hubbard broilers form 1 to 42 days of age under tropical condition. The starter diets (4-21d) and grower diets (22-42d) contained 20.1% and 17.0% protein; 2,750 and 2,780 kcal/kg ME, respectively. Diets were supplemented with DL-Met to provide total Met+Cys level ranging from 0.64 to 0.89% and 0.54 to 0.79% (six increments) in starter and grower diets, respectively. The other amino acids were calculated to meet or exceed the minimum level of NRC (1994) recommendation. They concluded that the requirement of total Met plus Cys during 4-21 and 22-42 days of age were 0.75 and 0.67%, respectively. In addition, Chamruspollert et al. (2004) studied the requirement of Met in Ross 208 broilers during 7-21 days of age by using corn-soy basal diets contained 23% protein (0.35% Met), 3,200 kcal/kg ME with supplemented of six levels of dietary Met (0, 0.05, 0.1, 0.15, 0.2, and 0.3%). They found that the Met requirement for young chicks is 0.43% that less than 0.50% which was suggested by NRC (1994). Whereas, Ojano-Dirain and Waldroup (2002) reported that optimum Met level during 22-42d of male Cobb-500 broiler was 0.44% higher than 0.38% which was recommended by NRC (1994). However, this level was closely to 0.43% which was suggested by Cobb guide (Cobb Vantress, 2008). Therefore, from the previous studies, it is possible that the NRC recommended level is adequate for 1-21d period, but not for 22-42d period.

2.3.2. Lysine requirement

Lysine is considered as the second limiting amino acid in broiler using corn-soybean diets following Met. Lys is determined as an important factor which affects performance and carcass quality of growing chick (Rezaei et al., 2004). Lys has no precursor role in the body and its utilization is only for protein accretion (Ojano-Dirain and Waldroup, 2002). In addition, Lysine has been used as the basis to evaluate the

requirement of other indispensable amino acids for ideal amino acid balance because almost of its content in the diet is used for protein synthesis (Samadi and Frank, 2007).

Labadan et al. (2001) indicated that Lysine requirement of male chicks (Ross male x Avian female) from 1 to 56 days of age. Dietary requirement for Lys was estimated by broken-line regression analysis of responses to six or seven dietary Lys levels. Dietary crude protein levels were 22, 21, and 20% in three consecutive experiments from 0-14, 15-28, and 22-42 days of age, respectively. All other amino acids were calculated to meet or exceed the minimum level of NRC (1994) recommendation. The dietary energy was maintained at 3,200 kcal/kg ME in all periods of study. The results showed that the Lys requirements during 0-14d and 15-28 days of age were 1.32 and 1.21%, respectively. These levels are higher than 1.10% which recommended by NRC (1994) for 1-21 days of age. The Lys requirement during 22-42 days of age was 0.99% that slightly less than 1.00% which suggested by NRC (1994). The study in male Cobb broilers by increasing dietary Lys from 1.03 to 1.12% during 22-42 days of age gave a higher breast meat yield and lower abdominal fat pad (Ojano-Dirain and Waldroup, 2002), while Cobb (2008) recommendation was 1.05% Lysine for 22-42 days of age. Therefore, from the previous studies the Lys requirement is higher than the suggestion level by NRC (1994).

2.3.3. Threonine requirement

Threonine is considered as the third essential amino acid following Met and Lys in practical broiler diets. It participates in protein synthesis, and its catabolism generates numerous products that important in metabolism such as pyruvate and Gly used for energy and protein production, respectively (Kidd and Kerr, 1996). In addition, Thr plays an important role in feather synthesis, precursor of Gly and Ser synthesis, gastrointestinal mucin production, and immune responses (Ojano-Dirain and Waldroup, 2002).

Zaghari et al. (2011) estimated standardized ileal digestible (SID) Thr requirement in Ross 308 male broilers from 1-21 days of age. Experimental treatments consisted of 8 levels of Thr (0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, and 1.1%) and 2 levels of protein (17.5 and 20.5%). The corn-soy diets were maintained equal energy at 3,100 kcal/kg ME. Fitted broken lines showed break points at 0.62 and 0.66% SID Thr for weigh gain at 17.5 and 20.5% protein, respectively.

Ayasan et al, (2009) studied the Thr requirement of Ross 308 male broilers from 22-42 days of age. Birds were fed a common grower diet (1-21d) containing 23% protein, 3,200 kcal/kg ME, 0.81% Thr, and 1.24% Lys. Five experimental diets contain 0.70, 0.75, 0.80, 0.85, and 0.90% Thr with 20.2% protein, isoenergetic (3,200 kcal/kg ME) and formulated to meet NRC (1994) recommendation. A linear response to dietary Thr for final body weight, body weight gain, and FCR indicated that 0.75% Thr supported the best performance. They suggested that the current NRC (1994) recommendation of 0.74% Thr for 21-42 days old broilers was adequate to support growth performance.

The requirements of Thr recommended by NRC (1994) were 0.80 and 0.74% for 1-21 and 22-42 days of age, respectively. These levels are closely to the suggestion levels by Cobb Vantress (2008) that were 0.79, 0.74, and 0.72% for 0-10, 11-22, and 23-42 days of age, respectively.

2.3.4. Tryptophan requirement

Tryptophan is classified as the fourth essential amino acid (Rosa et al, 2001). It involved in several metabolic functions such as a structural component of all proteins, a precursor for serotonin and melatonin synthesis (Corzo et al., 2005).

The requirements of Trp recommended by NRC (1994) are 0.20 and 0.18% for 1-21 and 22-42 days of age, respectively. Rosa et al. (2001) studied the Trp requirement

in male Arbor Acres High Yield and Ross-308 broilers during 1-21 days of age fed with diet contained 23% protein and 3,340 kcal/kg ME. They found that the Trp requirement for weigh gain in both strains was 0.17%. Corzo et al, (2005) determined the dose-response of dietary Trp in male broilers (Ross x Ross 508) from 0-20 days of age. Using eight levels of Trp (0.13, 0.15, 0.16, 0.17, 0.19, 0.21, 0.23, and 0.25%) with isoenergetic (3,100 kcal/kg ME) and equal protein levels (20% protein). They found that Trp requirements for weigh gain, FI, and FCR were 0.21, 0.20, and 0.22%, respectively. In terms of male Cobb broilers, Cobb Vantress (2008) recommended around 0.20 and 0.19% for 1-10 and 11-42 days of age, respectively. Therefore, dietary Trp levels recommended by NRC (1994) and Cobb Vantress (2008) were very closely and sufficiently for growth performance.

2.4 Protein reduction on growth performance and fecal nitrogen excretion

Reducing protein from 22.0 to 20.0% and 20.6 to 18.2% during 1-21 and 22-42d of age with constant amino acid levels can help to reduce nitrogen excretion in the feces 17.6% (from 51.0 to 42.0 g/kg feces) without negative effect on growth performance (Ferguson et al., 1998a). Kamran et al. (2004) determined the effect of decreasing dietary protein levels from 23.0 to 20.0% by maintaining optimal essential amino acids profile and ME on the growth performance of Hubbard broilers in hot climatic conditions for 42 days. They concluded that dietary protein could be reduced from 23 to 20% with beneficial effects on growth performance, carcass characteristics and economic returns. However, from the study of Kamran et al. (2008) in the same broiler strain, showed negative effects on growth performance when reducing dietary protein together with energy to maintain ME:CP ratio but constant essential amino acid levels. Therefore, this study used the concept that maintaining ME level in all diet, while decreasing CP levels with supplemented by synthetic amino acids which showed the better performance as mentioned above.

2.5 Cassava

Cassava or tapioca (*Manihot esculenta* Crantz) is one of the most important economic crops of Thailand. Cassava has ability to growth in poor quality soil and drought condition. Its production was around 4.0 to 9.6 tonnes/rai, depending on variety and cultivation practice. Almost 70 percent of world's cassava production comes from five countries, namely Nigeria, Brazil, Thailand, Indonesia, and the Congo Democratic Republic (Chauynarong et al., 2009).

The major source of energy in commercial broiler diet in Thailand has been known as corn. The demand of using corn as a feedstuffs tend to increase due to the increasing of broiler production, while its production tends to decrease every year (Office of Agricultural Economics, 2010). In contrast, cassava product and production per rai tend to increase every year (figure 2.2). It has been known that cassava is rich in carbohydrates but low in protein, amino acids and other nutrients. Therefore, it is used as source of energy for partially substitute for the main energy sources such as corn. Nutritive values of cassava root meal contains 3,500 kcal/kg ME for poultry, 2.0% protein, and minimal levels of numerous minerals such as 0.18% Ca, 0.10%P, 0.37% Mg, 0.86% K, 21ppm Mn, 190ppm Fe, 10ppm Zn, and 5ppm Cu (Khajarern et al, 1982). According to low nutritive values, high in hydrocyanic acid (HCN) and other physical problems i.e. dustiness as well as bulkiness, cassava meal was unpopular for use as animal feed ingredient in the past (Saentaweesuk et al., 2000) due to the physical problems (dustiness and bulkiness). However, shortage of corn due to rapid increasing of broiler farming and other use such as ethanol production will be a serious issue in the near future. Therefore, use of corn as main energy source of broiler feed is increasingly unjustified in economic terms. To solve this problem, there is a need to exploit cheaper energy sources to reduce feed cost. The comparison of nutritive values between cassava and corn are shown in Table 2.2. Cassava has higher energy (3,500 vs 3,370 kcal/kg ME) but lower protein (2 vs 8% protein) compared with corn (Kanto and Juttupornpong, 2004). However, cassava price is lower than corn (Figure 2.3).

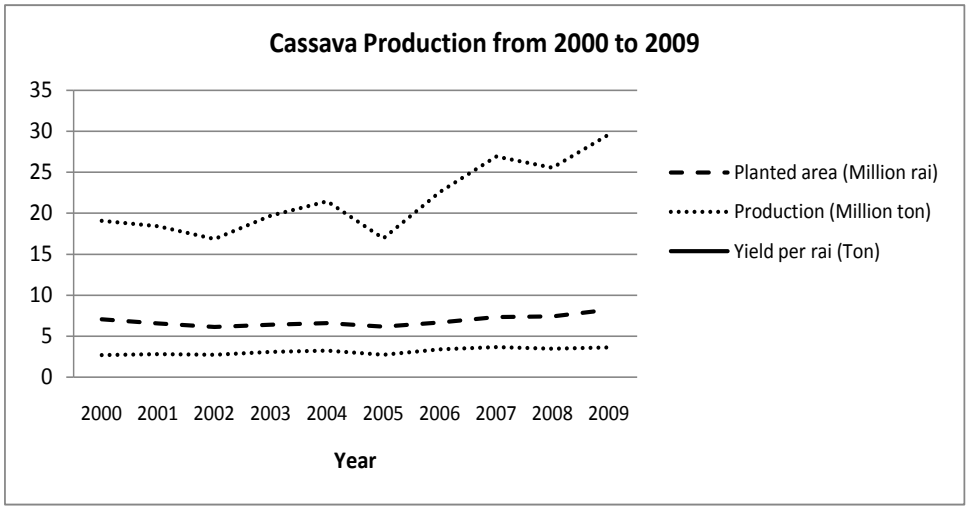


Figure 2.2 Thailand cassava production from 2000 – 2009 (Office of Agricultural Economics, 2010)

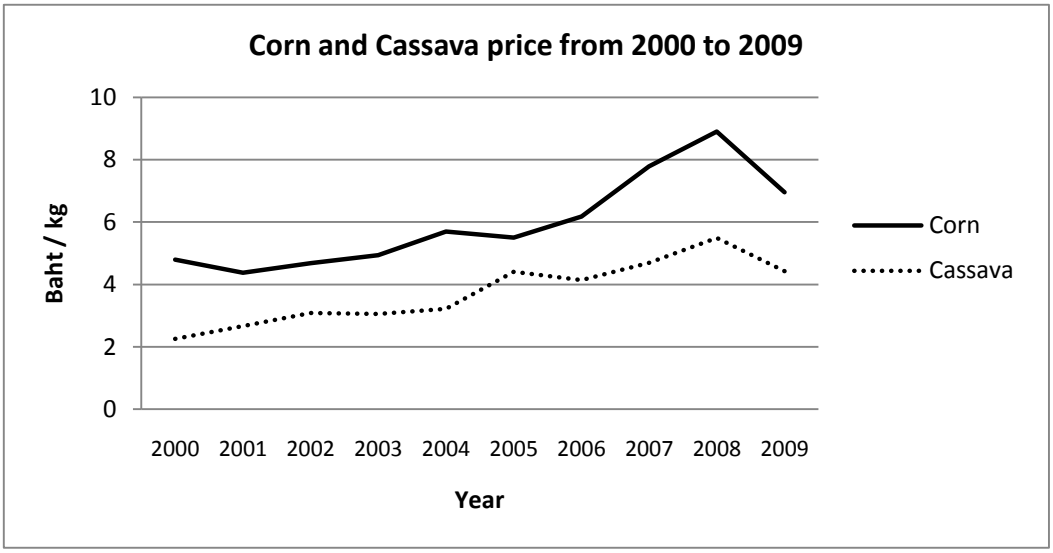


Figure 2.3 Thailand corn and cassava price from 2000 – 2009 (Office of Agricultural Economics, 2010)

Hydrocyanic acid (HCN) is the most concerned issue in using of cassava in animal diets. Garcia (1999) reported that total concentration of HCN in the diet should less than 50mg/kg diet. When animals receive HCN more than 50mg, it will substitute for oxygen in hemoglobin result in a problem of oxygen transportation and damaging of central nervous system. Animals that receive a minimum level of HCN for a period of

time, they will have chronic toxicity such as abnormal of central nervous system, goiter, poor growth rate. In addition, when cassava chip was dried by sunlight for 3-4 days, HCN will be reduced to the safety level for animals. Khajarern et al. (1982) reported that dried cassava chip up to 6 days, HCN would be reduced from 111.83 to 22.97mg/kg and was safe to fed animals.

Table 2.2 Nutritive value of cassava and corn

Nutritive value	Cassava	Corn
Protein (%)	2.00	8.00
Lysine (%)	0.09	0.25
Methionine(%)	0.03	0.19
Methionine + Cystine(%)	0.06	0.39
Tryptophan(%)	0.02	0.09
Threonine(%)	0.07	0.32
Isoleucine(%)	0.07	0.34
Arginine(%)	0.12	0.40
Leucine(%)	0.12	1.17
Phenylalanine + Tyrosine(%)	0.12	0.81
Histidine(%)	0.03	0.25
Valine(%)	0.09	0.46
Glycine(%)	0.08	0.33
ME poultry (kcal/kg)	3,500	3,370
Fat(%)	0.75	4.00
Calcium(%)	0.12	0.01
Phosphorus(%)	0.05	0.10
Fiber(%)	4.00	2.50

Kanto and Juttupornpong (2004)

Saentaweesuk et al. (2000) demonstrated the effect of substitution cassava for corn (0, 50, 75, and 100%) in broiler diet from 0-49 days of age. The experiment was designed into 3 periods of diets: 1) starter diets (1-21d) contained 0, 20.80, 31.20, 40.10% cassava and 4 different Lys levels (1.34, 1.38, 1.41, and 1.43%) with ME in the

range of 3,019 to 3,075 kcal/kg ; 2) the grower diets (22-42d) contained 0, 23.50, 35.34, and 47.65% cassava, 3,052 to 3,112 kcal/kg ME and 4 different Lys levels (1.28, 1.33, 1.36, and 1.38%); 3) the finisher diets (43-49d) contained 0, 27.96, 41.95, and 55.59% cassava, 3,142 to 3,225 kcal/kg ME and 4 different Lys levels (1.01, 1.07, 1.10, and 1.13%). While other essential amino acids (EAA) are maintained at the same levels in diets which are equal or above NRC (1994) recommendation. They indicated that substitution cassava for corn up to 50% has no negative effect on growth performance in all periods. Furthermore, mortality rate was lower compared with 100% corn diet because cassava had lower risk of mycotoxin contamination.

Due to the limitation of previous researches which studied in both reduction of protein and energy sources in the same time, therefore, the objective of the present study was to determine the optimum level of protein reduction in both corn and cassava substitute for corn 50% in the diets on growth performance, economic returns, ileal protein digestibility and nitrogen excretion in the feces.

CHAPTER III
MATERIALS AND METHODS

3.1 Animals and management

Total 2,688 day old male broilers (Cobb 500) were obtained from commercial hatchery after being vaccinated against Newcastle Disease, Infectious bronchitis and Infectious bursal disease (via coarse spray at hatch). Birds were randomly separated into 8 groups with 6 replicates of 56 birds each. A completely randomized experimental design with 2 x 4 factorial arrangement (2 energy sources and 4 protein levels), was applied. Initial weights of birds in each group were not significant difference. Birds were raised in an environmentally controlled house with 48 floor pens (200 x 200 x 90cm or 16.5 birds/m^2). The temperature was maintained at 35^{o}C for the first 3 days, and decreased by 2^{o}C every 3 days until 24^{o}C was reached. Chicks were subjected to artificial fluorescent illumination for 23h/d for the first 3 days, and decreased by 1h each day until 18h/d was reached. The whole experimental periods were divided into 2 phases: grower (1 to 21 d), and finisher period (22 to 42 d). Each pen was equipped with feeders and nipple drinker to provide free access to feed and water during the entire rearing period. This study was approved by Animal Care and Use Committee of the Faculty of Veterinary Science, Chulalongkorn University. The experimental trial was conducted on April, 2010.

3.2 Diets and Treatments

Experimental treatments consisted of 8 diets with 4 protein levels (100%, 95%, 90%, and 85% of Cobb (2008) recommendation and 2 energy sources (Corn; CO and Corn & Cassava; CC). The diets of first phase contained 19.0, 18.05, 17.10, and 16.15% protein with isocaloric at 3,083 kcal/kg ME (Table 3.1) while the diets of second phase contained 18.0, 17.10, 16.20, and 15.30% protein with isocaloric at 3,176 kcal/kg ME (Table 3.2). The low protein diets were supplemented with synthetic Met, Lys, Thr, and Trp to maintain the 4 primary limiting EAA levels to meet or exceed the minimum levels

of Cobb (2008) recommendation. The experimental diets were fed as pellet form except the diets used for determination the ileal protein digestibility were fed in mash form.

Table 3.1 Ingredients composition of the experimental diets in grower period (1-21d)

Treatment	T1 (100%CO)	T2 (95%CO)	T3 (90%CO)	T4 (85%CO)	T5 (100%CC)	T6 (95%CC)	T7 (90%CC)	T8 (85%CC)
Ingredient				%				
Corn, Grain	50.00	50.00	50.00	50.00	25.00	25.00	25.00	25.00
Cassava	0.00	0.00	0.00	0.00	25.00	25.00	25.00	25.00
Rice Bran	16.92	20.28	23.49	26.93	14.15	17.47	20.78	24.20
Rice bran ext	2.00	2.00	2.00	2.00	1.00	1.00	1.00	1.00
SBM 48%	17.35	14.21	11.15	7.95	21.57	18.44	15.35	12.16
Fish meal 56%	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
FFSB	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Palm Oil	2.55	2.17	1.83	1.42	2.12	1.74	1.37	0.97
Salt	0.27	0.27	0.27	0.27	0.29	0.29	0.29	0.29
MDCP	1.13	1.13	1.12	1.10	1.25	1.25	1.25	1.25
Limestone	0.98	0.98	1.03	1.03	0.86	0.88	0.88	0.88
Premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.17	0.19	0.22	0.25	0.21	0.24	0.26	0.29
L-Lysine	0.11	0.21	0.29	0.39	0.03	0.13	0.22	0.31
L-Threonine	0.02	0.06	0.10	0.15	0.02	0.06	0.10	0.15
L-Tryptophan	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Cost (฿/kg)	12.66	12.60	12.53	12.47	12.15	12.05	11.95	11.85

[1]Vitamin and mineral premix (per kg of diet): vitamin A (retinyl acetate), 11.023 IU; vitamin D (Cholecalciferol), 118 IU; vitamin E (DL-**α**-tocopheryl acetate), 23.54 IU; menadione (menadione dimethylpyrimidinol), 1.47 mg; B12, 0.00151 mg; riboflavin, 5.895 mg; niacin, 42.93 mg; D-pantothenic acid, 12.11 mg; choline (choline chloride), 477.7 mg; folic acid, 1.15 mg; pyridoxine (pyridoxine hydrochloride), 4.17 mg; thiamin (thiamin mononitrate), 1.23 mg; D-biotin, 0.075 mg; Mn ($MnSO_4 \cdot H_2O$), 110.60; Zn ($ZnSO_4 \cdot 7H_2O$), 110.40; Fe ($FeSO_4 \cdot 7H_2O$), 50; Cu ($CuSO_4 \cdot 5H_2O$), 8.30; I [Ca $(IO_3)2 \cdot 5H_2O$], 1.08; Se, 0.30; Co, 0.1; Mo, 0.05; a minimum of 6.98 mg of Ca and a minimum of 8.20 mg of calcium per kilogram of diet. The carrier was calcium carbonate, and the premix contained less than 0.7% mineral oil.

Table 3.2 Ingredients composition of experimental diets in finisher period (22-42d)

Treatment	T1 (100%CO)	T2 (95%CO)	T3 (90%CO)	T4 (85%CO)	T5 (100%CC)	T6 (95%CC)	T7 (90%CC)	T8 (85%CC)
Ingredient				%				
Corn, Grain	50.00	50.00	50.00	50.00	25.00	25.00	25.00	25.00
Cassava	0.00	0.00	0.00	0.00	25.00	25.00	25.00	25.00
Rice Bran	20.31	23.38	26.57	29.80	16.19	19.38	22.71	25.80
SBM 48%	14.88	11.95	8.98	5.94	19.18	16.22	13.20	10.22
Fish meal 56%	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
FFSB	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Palm Oil	3.80	3.47	3.10	2.73	3.63	3.25	2.80	2.53
Salt	0.24	0.24	0.24	0.24	0.27	0.27	0.27	0.27
MDCP	1.03	1.03	1.03	1.03	1.15	1.15	1.10	1.10
Limestone	0.90	0.93	0.93	0.93	0.77	0.77	0.80	0.80
Premix[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.17	0.20	0.22	0.25	0.22	0.24	0.27	0.29
L-Lysine	0.13	0.22	0.31	0.40	0.05	0.14	0.23	0.32
L-Threonine	0.04	0.08	0.12	0.16	0.04	0.08	0.12	0.16
L-Tryptophan	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.01
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Cost (฿/kg)	12.75	12.73	12.71	12.69	12.28	12.22	12.16	12.09

[1]Vitamin and mineral premix (per kg of diet): vitamin A (retinyl acetate), 11.023 IU; vitamin D (Cholecalciferol), 118 IU; vitamin E (DL-**α**-tocopheryl acetate), 23.54 IU; menadione (menadione dimethylpyrimidinol), 1.47 mg; B12, 0.00151 mg; riboflavin, 5.895 mg; niacin, 42.93 mg; D-pantothenic acid, 12.11 mg; choline (choline chloride), 477.7 mg; folic acid, 1.15 mg; pyridoxine (pyridoxine hydrochloride), 4.17 mg; thiamin (thiamin mononitrate), 1.23 mg; D-biotin, 0.075 mg; Mn ($MnSO_4 \cdot H_2O$), 110.60; Zn ($ZnSO_4 \cdot 7H_2O$), 110.40; Fe ($FeSO_4 \cdot 7H_2O$), 50; Cu ($CuSO_4 \cdot 5H_2O$), 8.30; I [Ca $(IO_3)2 \cdot 5H_2O$], 1.08; Se, 0.30; Co, 0.1; Mo, 0.05; a minimum of 6.98 mg of Ca and a minimum of 8.20 mg of calcium per kilogram of diet. The carrier was calcium carbonate, and the premix contained less than 0.7% mineral oil.

3.3 Data and Sample Collection

The temperature and relative humidity were recorded daily at 08:00, 13:00, and 17:00 h throughout the experimental period. The average, minimum, and maximum temperatures were 28.8 ± 0.31, 26.5, and 33.5 ^{0}C, while the average, minimum, and maximum relative humidity were 69.1 ± 0.92, 43.2 , and 86.4%, respectively. Chicks were grouping weighed at 1, 21 and 42d of age. The feed intake was recorded during days 1 to 21 and days 22 to 42. Dead and cull chicks due to the leg problems were recorded daily for calculating mortality rate. Growth performance and economic return were calculated by the method of Suvanatad (2003) as follows:

Growth performance:

$$\text{Average daily growth; ADG (g/d)} = \frac{\text{Final weight (g) – initial weight (g)}}{\text{Numbers of rearing day}}$$

$$\text{Feed conversion rate; FCR} = \frac{\text{Feed Intake (g)}}{\text{Weight gain (g)}}$$

Economic returns:

$$\text{European Efficiency Factor; EEF} = \frac{\text{\% livability x average body weight (kg) x 100}}{\text{Selling age (d) x FCR}}$$

$$\text{Feed cost / kg chick production (฿/kg)} = \frac{\text{Feed intake (kg) x feed cost / kg (฿)}}{\text{Total live weight of chickens (kg)}}$$

Salable chicken weight / rearing area

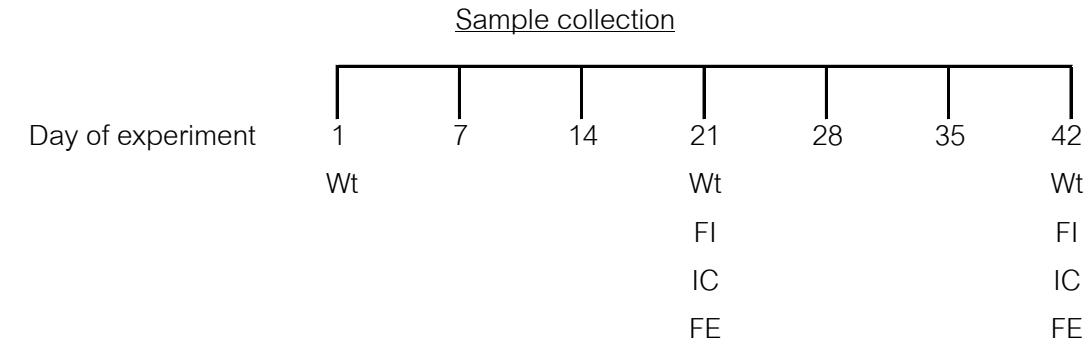
$$= \frac{\text{Total live weight of chickens (kg)}}{\text{Rearing area } (m^2)}$$

Income / square meter

= Salable chick weight / m^2 x [Chick cost (฿/kg) – Feed cost (฿/kg)

On the days 18 and 39 of the experiment, eight and six chicks in each pen were randomly selected into separated pens, respectively. Those chicks were continuously fed the same diets as their main groups with supplementation of chromic oxide 0.25% for four days (Steenfeldt et al., 2003). On the days 21 and 42 of experiment, those chicks were put together in clean plastic based box of each pen for 30 minutes, and then they were weighed and anesthetized by overdose injection of sodium pentobarbital (100 mg/kg) into jugular vein. The unconscious chicks were killed and ileal content was individually collected from Meckel's diverticulum to ileocaecal junction; their excreta were collected from each box. The collected contents were weighed equally base on the lowest weight of their contents, and then the contents were blended together to obtain the ileal content sample of each pen. Both ileal contents and fecal samples were dried at 55^{o}C for 48 hours and stored at -20^{0}C for further analysis. The ileal protein digestible coefficient was calculated as the following equation adopted form Short et al., (1999). Schedule of sample collection plan were shown in Figure 3.1.

$$\text{Protein digestible coefficient} = \frac{\%\ Cr_2O_3 \text{ in feed}}{\%\ Cr_2O_3 \text{ in digesta}} \times \frac{\%\ \text{Protein in digesta}}{\%\ \text{Protein in feed}}$$



Where:

Wt	= chick weighing
FI	= feed intake record
IC	= ileal content collection
FE	= feces collection

Figure 3.1 Diagram showing the whole period of sample collection

3.4 Chemical Analysis

Two batches of cassava pellets used in the experiment were randomly collected and sent to SGS (Thailand) Limited laboratory for HCN analysis according to the method number 915.03 (AOAC, 1995) before mixing feed. All diets were analyzed for nutritional content by proximate analysis (AOAC, 1990). Amino acid (AA) profiles in the diets were analyzed using an amino acid analyzer (Biochrom-30, Biochrom Ltd., UK) by the methods described by the commission of the European communities as Commission directive 98/64/EC of 3 September 1998. Na, Ca and P in the diets were analyzed by the method number 969.23, 927.02, and 965.17 (AOAC, 2006), respectively. Experimental diets which mixed with chromic oxide, ileal contents and feces were analyzed for chromic oxide (Williams and David, 1962), while dry matter, organic matter, nitrogen and protein were analyzed according to the methods of AOAC (1990).

Determination of Na, Ca, and P

Sodium (Na) in the diets was determined using flame absorption method (method number 969.23) as described by AOAC (2006) as follows: preparing Sodium standard solutions (1mg Na/mL), dried reagent grade of NaCl 2 h at 110 ^{0}C, then cooled it down in desiccators and weighed it 2.5421 g into 1 L volumetric flask and diluted to volume with H_2O. Afterward, preparing working solution (0.00003, 0.0001, 0.0003, and 0.005 mg Na/mL) by pipet 1 mL Na stock solution into 100 mL volumetric flask and diluted to volume with H_2O. Pipetted 0.3, 1.0, 3.0, and 5.0 mL diluted stock solution into separated 100 mL volumetric flasks and diluted to volume with H_2O. After that, preparing test sample by weighing 4 g diet into crucible, and burned on electric hot plate. Then, placed it in furnace and bring to 525 ^{0}C for 2 h until white ash appeared, cooled it down and weighed. Added 15 mL diluted HNO_3 to crucible, breaking up ash with stirring rod if necessary and filtrated through acid-washed quantitative paper into 100 mL volumetric flask. The residue and paper were washed 3 times with H_2O. Then, placed 1 mL aliquot in 100 mL volumetric flask and diluted to volume with H_2O. After that, blank solution was prepared by diluting of 2 mL HNO_3 to 100 mL with H_2O. Finally, read blank, standards, and test solution by flam photometers at 589 nm.

Calcium (Ca) in the diets was determined using method number 927.02 (AOAC, 2006). The process was described below: approximately 2 g finely ground diet were weighed into porcelain dish and ignited in furnace to C-free ash. The residue was boiled in 40 mL HCl and few drops HNO_3. Then, the solution was moved to 250 mL volumetric flask, cooled down, diluted to volume, and mixed thoroughly. Pipetted 25 mL clear liquid into beaker and diluted to ca 100 mL, and added 2 drops methyl red. Added NH_4OH dropwise to pH 5.6, as shown by intermediate brownish-orange (if overstepped, added HCl with dropper to orange). Added 2 more drops HCl, color should now be pink (pH 2.5-3.0), not orange. Diluted to ca 150 mL, brought to boil, and slowly added, with constant stirring, 10 mL hot saturated (4.2%) solution of $(NH_4)_2C_2O_4$. If red changed to orange or yellow, added HCl dropwise until color again changed to pink, let stand

overnight for precipitated to settle. The supernatant was be filtrated through quantitative paper, and precipitate was washed thoroughly with NH_4OH. Placed paper or crucible with precipitate in original beaker, and add mixture of 125 mL H_2O and 5 mL H_2SO_4. Heat to 70°C and titrated with 0.02M $KMnO_4$ to first slight pink. Correction for blank and calculated percent Ca (1 mL 0.02M $KMnO_4$ = 2.004 mg Ca)

Phosphorus (P) in the diets was determined using method number 965.17 (AOAC, 2006). Preparing molybdovanadate reagent, dissolved 40 g ammonium molybdate·$4H_2O$ in 400 mL hot H_2O and cooled it down. Dissolved 2 g ammonium cetavanadate in 250 mL hot H_2O, cooled down and added 250 mL 70% $HClO_4$. Gradually added molybdate solution to vanadate solution with stirring, and diluted to 2 L. Preparing Phosphorus standard solution, for stock solution (2 mg P/mL), dissolved 8.788 g KH_2PO_4 in H_2O and diluted to 1 L, and for working solution (0.1 mg P/mL), diluted 50 mL stock solution to 1 L. Then, prepare standard curve, aliquots of working standard solution containing 0.5, 0.8, 1.0, and 1.5 mg P were moved to 100 mL volumetric flasks. After that, 2 g diet in 150 mL beaker were burned for 4 h at 600 ^{0}C. Then, cooled it down, added 40 mL HCl and several drops HNO_3, and brought to boiling point. Cooled down, moved to 200 mL volumetric flask, and diluted to volume with H_2O. Aaliquot containing 0.5-1.5 mg P were filtrated and placed in 100 mL volumetric flask. Then, 20 mL molybdovanadate reagent was added and diluted to volume with H_2O, and mixed well. Let stand 10 min; then read %T at 400 nm against 0.5 mg standard set at 100% T. (Use 15 mm diameter cells). Finally, mg of P was determined form standard curve.

$$P, \% = \frac{\text{mg P in aliquot}}{\text{g diet in aliquot x 10}}$$

Determination of Chromic oxide

Chromic oxide in diets, ileal content and feces were determined as the method described by Williams and David (1962). Phosphoric acid – manganese sulfate solution was prepared by mixing 30 mL of 10% w/v $MnSO_4 \cdot 4H_2O$ with 1 L 85% phosphoric acid. To prepare Potassium bromated solution, 4.5 g $KBrO_3$ was dissolved in 100 mL distilled H_2O. Calcium chloride solution (4,000 ppm) was prepared by merging 14.68 g $CaCl_2 \cdot 2H_2O$ in H_2O, and made up volume to 1 L in volumetric flask. After all of solutions were prepared, 1g sample was accurately weighed into porcelain crucible. Then it was heated at 100-200 ^{0}C until free of smoke, ignited at 600 ^{0}C for 3 h in muffle furnace and cooled down. Ash was moved to 150 mL beaker, added 3 ml phosphoric acid solution, ash was wet with the acid solution and then 4 mL $KBrO_3$ solution was used to wash down the side of the beaker and covered with a watch glass and placed on a preheated hot plate, under a hood, boiled gently until a purple color appeared, did not boil dry. The residue was washed from beaker to 100 mL volumetric flask with H_2O. Then, 12.5 ml $CaCl_2$ solution was added, brought to volume with H_2O and mixed well. Afterward, 10 mL clear solution was added into 100 mL volumetric flask and made volume with H_2O mixed well. Then, standard solution was prepared, one chromium-free (blank) sample was used to provide a mineral matrix background for the standard solution. A typical curve should contain the following point: 1, 2, 3, 4, and 5 ppm. To attain these, 10 mL of blank solution was added and brought to volume with H_2O. If dilution rate was changed for the samples then an equivalent changed in the volume of blank added would be needed for the standard. Finally, the concentration of Cr was determined using Atomic Absorption Spectrophotometer (AAS) at 357.9 nm and calculated percentage of chromium.

$$\% \text{ Cr} = \frac{\text{ppm from AAS}}{\text{(mL sample solution used) x g sample}}$$

3.5 Statistical analysis

The growth performance data, economic returns, ileal protein digestibility, and nitrogen excretion in feces were analyzed using one-way analysis of variance (ANOVA) to determine the effects of energy source and protein levels. Significant differences among means were compared with least significant difference (LSD) test. The level of significant difference was set at $P < 0.05$.

CHAPTER IV
RESULTS

The chemical analysis of HCN levels contained in two batches of cassava pellets used in this experiment were less than 5 and 6.8 mg/kg. Nutrient compositions in all experimental diets during grower (1-21d) and finisher (22-42) periods were shown in Table 4.1 and 4.2, respectively. Analyzed protein values for all diets in both periods were mostly in agreement with the calculated values. Nonetheless, there were some analyzed AA values that slightly lower than calculated values in both periods especially Met plus Cys (M+C).

In grower diets (Table 4.1), the analyzed M+C values varying from 0.75 to 0.80 while the calculated value was 0.84. Other AA levels that slightly lower than calculated values were Lys in 100%CO diet (1.09 vs 1.10%); Thr in 100, 95, and 85%CC diets (0.68, 0.73, 0.72 vs 0.74); and Trp in 85%CO diet (0.18 vs 0.19%), respectively.

In finisher diets (Table 4.2), all of the analyzed M+C values were lower than calculated values varying from 0.78 to 0.81 vs 0.82%. Analyzed Lys in 100%CO, 100 and 95%CC diets were slightly lower than calculation (1.04, 1.02, 1.02 vs 1.05%). Threonine in all CO and 95%CC diets were slightly less than requirement varying from 0.67 to 0.70 compared with required level at 0.72%. Besides, analyzed Trp values in 85% protein of both CO and CC diets were inferior to the requirements (0.17, 0.18 vs 0.19), respectively.

Table 4.1 Chemical analysis of experimental diets in grower period (1-21d)

Treatment	T1 (100%CO)	T2 (95%CO)	T3 (90%CO)	T4 (85%CO)	T5 (100%CC)	T6 (95%CC)	T7 (90%CC)	T8 (85%CC)
Nutrient composition (%)								
GE (kcal/kg)	4,170	4,197	4,194	4,163	4,086	4,071	4,055	4,015
CP	19.1	18.0	17.2	16.4	19.3	18.1	17.5	16.6
Fat	8.5	8.39	8.62	8.84	6.65	6.7	6.27	7.02
Fiber	2.57	2.73	2.81	2.85	3.48	3.48	3.62	3.73
Ash	6.70	6.80	6.80	6.90	7.50	7.55	7.65	7.60
Moisture	10	10.1	9.95	10	9.85	9.8	9.9	10
Ca	0.99	1.00	1.04	1.02	1.11	1.17	1.09	1.07
Total P	0.97	0.99	1.01	1.08	0.96	0.96	0.98	1.03
Na	0.19	0.18	0.18	0.19	0.23	0.23	0.23	0.20
Amino acids (%), DM basis								
Cys	0.29	0.30	0.29	0.28	0.28	0.27	0.26	0.26
Met	0.46	0.48	0.49	0.51	0.53	0.52	0.53	0.54
M+C	0.75	0.79	0.78	0.79	0.80	0.79	0.80	0.79
Lys	1.09	1.13	1.12	1.12	1.14	1.12	1.15	1.16
Thr	0.68	0.73	0.74	0.72	0.75	0.76	0.76	0.75
Trp	0.24	0.21	0.20	0.18	0.23	0.22	0.20	0.19
Asp	1.78	1.85	1.71	1.56	1.49	1.83	1.73	1.63
Ser	0.89	0.91	0.87	0.80	0.77	0.88	0.83	0.80
Glu	3.30	3.31	3.10	2.90	2.73	3.16	2.99	2.83
Gly	0.91	0.93	0.86	0.84	0.81	0.90	0.85	0.82
Ala	1.01	1.03	0.97	0.94	0.92	0.95	0.92	0.90
Val	0.90	0.93	0.84	0.80	0.78	0.84	0.83	0.79
Ile	0.76	0.77	0.68	0.64	0.61	0.73	0.70	0.64
Leu	1.52	1.54	1.45	1.36	1.31	1.39	1.33	1.26
Phe	0.98	0.96	0.90	0.86	0.82	0.94	0.89	0.86
His	0.49	0.51	0.47	0.44	0.43	0.46	0.44	0.42
Arg	1.23	1.28	1.19	1.12	1.09	1.22	1.17	1.12
Pro	1.09	1.17	1.14	1.06	1.01	0.97	0.93	0.90

* % of Cobb 500 requirement during 1-21 days of age (Cobb, 2008): 3,038 kcal/kg ME, protein 19, Ca 0.96, available P 0.48, Na 0.17, Lys 1.10, Met + Cys 0.84, Thr 0.74, Trp 0.19, Arg 1.17.

Table 4.2 Chemical analysis of experimental diets in finisher period (22-42d)

Treatment	T1 (100%CO)	T2 (95%CO)	T3 (90%CO)	T4 (85%CO)	T5 (100%CC)	T6 (95%CC)	T7 (90%CC)	T8 (85%CC)
Nutrient composition (%)								
GE (kcal/kg)	4,256	4,262	4,258	4,256	4,192	4,173	4,178	4,169
CP	18.2	17.4	16.5	15.5	18.0	17.4	16.3	15.6
Fat	10.60	10.50	10.40	11.00	8.62	9.06	9.31	9.41
Fiber	2.55	2.64	2.63	2.69	3.41	3.58	3.97	3.82
Ash	6.20	6.15	6.10	6.25	6.90	6.85	7.00	7.05
Moisture	11.2	11.1	11.0	11.0	10.4	10.4	10.2	10.2
Ca	1.05	1.02	1.03	1.12	1.11	1.11	1.06	1.06
Total P	0.96	0.99	0.98	1.00	0.92	0.93	0.93	1.00
Na	0.19	0.19	0.18	0.20	0.21	0.21	0.20	0.20
Amino acids (%), DM basis								
Cys	0.29	0.28	0.27	0.26	0.27	0.26	0.26	0.25
Met	0.49	0.49	0.51	0.53	0.51	0.53	0.54	0.55
M+C	0.78	0.78	0.78	0.79	0.78	0.78	0.80	0.81
Lys	1.04	1.07	1.06	1.08	1.02	1.02	1.06	1.08
Thr	0.70	0.70	0.67	0.69	0.99	0.69	0.92	0.82
Trp	0.20	0.19	0.19	0.17	0.20	0.20	0.20	0.18
Asp	1.68	1.59	1.42	1.32	1.85	1.65	1.66	1.52
Ser	0.86	0.81	0.74	0.69	0.64	0.82	0.65	0.64
Glu	3.06	2.92	2.63	2.48	3.09	2.89	2.78	2.59
Gly	0.95	0.92	0.85	0.86	0.93	0.88	0.91	0.87
Ala	1.00	0.97	0.92	0.91	0.95	0.90	0.91	0.87
Val	0.85	0.85	0.77	0.77	0.86	0.77	0.79	0.78
Ile	0.66	0.67	0.60	0.56	0.71	0.64	0.62	0.59
Leu	1.40	1.35	1.25	1.19	1.35	1.25	1.23	1.15
Phe	0.93	0.91	0.84	0.81	0.90	0.89	0.81	0.76
His	0.45	0.44	0.40	0.38	0.44	0.41	0.41	0.39
Arg	1.17	1.13	1.03	0.98	1.18	1.10	1.09	1.03
Pro	1.03	1.02	0.93	0.93	0.97	0.92	0.88	0.85

* % of Cobb 500 requirement during 22-42 days of age (Cobb, 2008): 3,176 kcal/kg ME, protein 18, Ca 0.90, available P 0.45, Na 0.16, Lys 1.05, Met + Cys 0.82, Thr 0.72, Trp 0.19, Arg 1.13.

4.1 Effect of protein levels and energy sources on growth performance.

The effect of various protein levels and energy sources on growth performance of broilers during 1-21 days of age was shown in Table 4.3. Initial body weight was not significant difference in all groups. There were no interaction between energy sources and protein levels and also no treatment effects on final body weight, ADG, feed intake and mortality among groups. However, FCR in 85% protein group was significantly higher than other protein groups ($P<0.05$) but not differ between energy sources. Birds fed 100 and 95%CC diets had lowest FCR (1.62) which was as same as those birds fed 90% and upper protein levels of CO diets, whereas bird fed 85% protein in both CO and CC diets had highest FCR (1.72 and 1.71, respectively) that was significantly ($P<0.05$) different from other diets. Energy sources did not influence on all parameters.

During 22-42 days of age (Table 4.4), the initial body weight was not different among groups. Final body weight ($P<0.0001$) and ADG ($P=0.0001$) in 85% protein group were significantly lower, whereas FCR ($P<0.0001$) was significantly higher than other protein groups. Feed intake and mortality were not different among protein groups. However, there was interaction between energy source and protein levels on feed intake ($P<0.05$). Energy sources had no effect on final body weight, ADG, FCR and mortality but affected on feed intake. Birds in CC group had significantly higher feed intake than CO group ($P<0.05$). Birds received 100% protein CO group showed highest final body weight (2,637.4 g/b) and ADG (78.3 g/b/d) but not significantly different from those birds fed 90 and 95% protein in CO group as well as those birds received all protein levels of CC group. Birds fed 90% protein in CC group had highest feed intake (3,308 g/b), whereas bird fed 85% protein in CO diet had lowest feed intake (2,894.9 g/b). Birds fed 95 and 100% protein in CC diets showed the best FCR (1.91), on the contrary bird fed 85% protein in CO and CC diets displayed the worst FCR (2.11 and 2.19, respectively).

Table 4.5 showed the growth data in the overall period (0-42 days of age). The effect of protein levels and energy sources on growth traits presented the same trend as

growth traits during 22-42 days of age. There was also interaction between energy sources and protein levels on feed intake ($P<0.05$). Overall mortality rate was lower than 3% and was not significant difference among treatment groups. In addition, birds in CC group had significantly higher FCR than CO group ($P=0.0067$). Birds fed 100% protein in CO group had the best final body weight (2,637.4 g/b), ADG (61.8 g/b/d), and FCR (1.65). Nonetheless, the responses of birds fed 90 to 95% protein in CO and 90 to 100% protein in CC group to final body weight and ADG were the same as 100% protein in CO group. Feed intake was highest in 90% protein in CC group (4,550.3 g/b), whereas the lowest was 85% protein in CO group (4,300.6 g/b). Bird fed 90-95% protein in CO and 95-100% protein in CC diets had the same FCR as those birds fed 100% protein in CO groups.

4.2 Effect of protein levels and energy sources on economic returns.

The effects of protein levels and energy sources on economic returns were shown in Table 4.6. There was no interaction between energy source and protein levels and also energy sources in all economic parameters. Birds fed 85% protein group had lowest EEF, bird weight/m^2, and income/m^2 but highest feed cost/kg bird compared with those birds in other protein groups ($P<0.0001$). Birds received 100% protein in CO diet had highest EEF (372.9) and bird weight/m^2 (27.2kg/m^2) that were not significantly different from those birds fed 90-95% protein in CO and 95-100% protein in CC diets. Feed cost per kg of live weight showed negative correlation with economic values. However, birds fed 95% protein in CC diet had lowest feed cost/kg of live weight (24.3฿/kg) and did not significantly differ from those birds fed 100% and 90-100% protein in CC and CO diets, respectively.

Table 4.3 Effect of protein levels and energy sources on growth performance in grower period (1-21d)

Treatment			Initial	Final	ADG	Feed	FCR	Mortality
Group	Energy source	Protein level (% Cobb Std.)	body wt (g/b)	body wt. (g/b)	(g/b/d)	Intake (g/b)		(%)
1	CO	100	43.0	849.3	38.4	1,293.9	1.61^{cd}	0.60
2	CO	95	42.9	858.8	38.9	1,291.3	1.59^{cd}	1.49
3	CO	90	42.9	860.9	39.0	1,269.3	1.57^{d}	1.49
4	CO	85	42.9	751.7	33.8	1,214.8	1.72^{a}	0.90
5	CC	100	42.7	835.3	37.7	1,282.1	1.62^{bcd}	1.49
6	CC	95	42.7	846.4	38.3	1,302.3	1.62^{bcd}	0.90
7	CC	90	42.7	816.0	36.8	1,296.0	1.68^{abc}	1.19
8	CC	85	42.8	811.7	36.6	1,309.9	1.71^{a}	1.19
SEM			0.06	10.73	0.51	9.77	0.01	0.16
CV, %			1.15	8.64	9.10	5.19	5.01	96.32
Source					*P-value*			
	Treatment diet		0.8881	0.1887	0.1867	0.3182	0.0210	0.7956
	Protein levels (A)		0.9883	0.0838	0.0826	0.6345	0.0091	0.8978
	Energy sources (B)		0.1145	0.8920	0.9006	0.1238	0.1327	0.8166
	(A) x (B)		0.9827	0.3381	0.3372	0.2478	0.3402	0.3789
Main effect								
Protein level								
	100%		42.8	842.3	38.1	1,288.0	1.61^{b}	1.04
	95%		42.8	852.6	38.6	1,296.8	1.60^{b}	1.19
	90%		42.8	838.5	37.9	1,282.7	1.62^{b}	1.34
	85%		42.9	781.7	35.2	1,262.4	1.71^{a}	1.04
Energy source								
	CO		42.9	830.2	37.5	1,267.3	1.62	1.12
	CC		42.7	827.3	37.4	1,297.6	1.65	1.19

*^{a,b,c,d} Means in a column with different superscripts are significantly different (P<0.05)

Table 4.4 Effect of protein levels and energy sources on growth performance in finisher period (22-42d)

Treatment			Initial	Final	ADG	Feed	FCR	Mortality
Group	Energy source	Protein level (% Cobb Std.)	body wt (g/b)	body wt. (g/b)	(g/b/d)	Intake (g/b)		(%)
1	CO	100	849.3	2,637.4^{a}	78.3^{a}	3,046.3^{cd}	1.85^{c}	2.09
2	CO	95	858.8	2,580.4^{ab}	75.2^{ab}	3,091.8^{bcd}	1.95^{bc}	1.40
3	CO	90	860.9	2,556.2^{ab}	74.2^{ab}	3,053.0^{cd}	1.96^{bc}	1.76
4	CO	85	751.7	2,243.6^{c}	65.2^{c}	2,894.9^{d}	2.11^{a}	1.75
5	CC	100	835.3	2,581.4^{ab}	76.5^{ab}	3,073.5^{cd}	1.91^{c}	1.05
6	CC	95	846.4	2,616.3^{a}	77.6^{a}	3,127.8^{abc}	1.91^{c}	1.40
7	CC	90	816.0	2,549.9^{ab}	76.0^{ab}	3,308.0^{a}	2.07^{ab}	2.11
8	CC	85	811.7	2,446.6^{b}	71.4^{b}	3,292.6^{ab}	2.19^{a}	2.11
SEM			10.73	25.08	0.86	29.94	0.02	0.17
CV, %			8.64	5.36	6.44	5.69	5.13	72.43
Source					*P-value*			
	Treatment diet		0.1887	0.0002	0.0006	0.0051	<.0001	0.7354
	Protein levels (A)		0.0838	<.0001	0.0001	0.4085	<.0001	0.6371
	Energy sources (B)		0.8920	0.2620	0.1165	0.0012	0.0895	0.8212
	(A) x (B)		0.3381	0.1207	0.2510	0.0374	0.3343	0.4696
Main effect								
Protein level								
	100%		842.30	2,609.4^{a}	77.4^{a}	3,059.9	1.88^{c}	1.57
	95%		852.60	2,598.4^{a}	76.4^{a}	3,109.8	1.93^{bc}	1.40
	90%		838.50	2,553.0^{a}	75.1^{a}	3,180.5	2.02^{b}	1.94
	85%		781.70	2,345.1^{b}	68.3^{b}	3,093.8	2.15^{a}	1.94
Energy source								
	CO		830.2	2,504.4	73.20	3,021.5^{b}	1.97	1.75
	CC		827.3	2,548.5	75.41	3,200.5^{a}	2.02	1.67

*^{a,b,c,d} Means in a column with different superscripts are significantly different (P<0.05)

Table 4.5 Effect of protein levels and energy sources on growth performance in overall period (1-42d)

Treatment			Initial	Final	ADG	Feed	FCR	Mortality
Group	Energy source	Protein level (% Cobb Std.)	body wt (g/b)	body wt. (g/b)	(g/b/d)	Intake (g/b)		(%)
1	CO	100	43.0	2,637.4^{a}	61.8^{a}	4,288.3^{abc}	1.65^{c}	2.38
2	CO	95	42.9	2,580.4^{ab}	60.4^{ab}	4,326.5^{ab}	1.70^{c}	2.68
3	CO	90	42.9	2,556.2^{ab}	59.8^{ab}	4,266.5^{bc}	1.69^{c}	2.98
4	CO	85	42.9	2,243.6^{c}	52.4^{c}	4,056.2^{c}	1.84^{ab}	2.38
5	CC	100	42.7	2,581.4^{ab}	60.4^{ab}	4,300.6^{abc}	1.69^{c}	2.68
6	CC	95	42.7	2,616.3^{a}	61.3^{a}	4,374.4^{ab}	1.70^{c}	2.09
7	CC	90	42.7	2,549.9^{ab}	59.7^{ab}	4,550.3^{a}	1.81^{b}	2.98
8	CC	85	42.8	2,446.6^{b}	57.2^{b}	4,547.4^{a}	1.89^{a}	2.98
SEM			0.06	25.08	0.59	37.10	0.01	0.15
CV, %			1.15	5.36	5.45	5.19	3.50	40.59
Source					*P-value*			
	Treatment diet		0.8881	0.0002	0.0002	0.0122	<.0001	0.7653
	Protein levels (A)		0.9883	<.0001	<.0001	0.5848	<.0001	0.5747
	Energy sources (B)		0.1145	0.2620	0.2635	0.0026	0.0067	0.8099
	(A) x (B)		0.9827	0.1207	0.1206	0.0442	0.1269	0.5752
Main effect								
Protein level								
	100%		42.8	2,609.4^{a}	61.1^{a}	4,294.5	1.67^{c}	2.53
	95%		42.8	2,598.4^{a}	60.9^{a}	4,350.5	1.70^{c}	2.38
	90%		42.8	2,553.0^{a}	59.8^{a}	4,408.4	1.75^{b}	2.97
	85%		42.9	2,345.1^{b}	54.8^{b}	4,301.8	1.86^{a}	2.68
Energy source								
	CO		42.9	2,504.4	58.60	4,234.4^{b}	1.72^{b}	2.61
	CC		42.7	2,548.5	59.66	4,443.2^{a}	1.77^{a}	2.68

*^{a,b,c,d} Means in a column with different superscripts are significantly different (P<0.05)

Table 4.6 Effect of protein levels and energy sources on economic returns

Treatment			EEF	Feed cost/ 1kg bird	Bird wt/ sq.m	Income/ sq.m
Group	Energy source	Protein level (% Cobb Std.)	(g/b)	(฿)	(kg/m^2)	(฿)
1	CO	100	372.9^{a}	25.2^{cd}	27.2^{a}	481.5^{ab}
2	CO	95	351.8^{ab}	25.4^{cd}	26.4^{ab}	461.2^{ab}
3	CO	90	350.8^{ab}	25.0^{cd}	26.3^{ab}	470.1^{ab}
4	CO	85	284.8^{d}	25.4^{cd}	23.1^{c}	350.2^{d}
5	CC	100	355.2^{ab}	24.6^{d}	26.5^{ab}	485.0^{ab}
6	CC	95	359.8^{a}	24.3^{d}	27.0^{a}	500.6^{a}
7	CC	90	328.4^{bc}	25.8^{bc}	26.2^{ab}	449.0^{bc}
8	CC	85	302.2^{cd}	26.6^{b}	25.2^{b}	409.6^{c}
SEM			5.23	0.20	0.25	8.51
CV, %			7.01	3.77	5.26	8.73
Source					*P-value*	
	Treatment diet		<.0001	<.0001	0.0002	<.0001
	Protein levels (A)		<.0001	<.0001	<0.0001	<.0001
	Energy sources (B)		0.5920	0.0780	0.2350	0.0809
	(A) x (B)		0.1279	0.0696	0.1089	0.0722
Main effect						
Protein level						
	100%		364.1^{a}	24.9^{b}	26.9^{a}	483.2^{a}
	95%		355.8^{ab}	24.9^{b}	26.7^{a}	480.9^{a}
	90%		339.6^{b}	25.4^{b}	26.2^{a}	459.6^{a}
	85%		293.5^{c}	27.2^{a}	24.2^{b}	379.9^{b}
Energy source						
	CO		340.1	25.8	25.8	440.7
	CC		336.4	25.3	26.2	461.1

*^{a,b,c,d} Means in a column with different superscripts are significantly different (P<0.05)

4.3 Effect of protein levels and energy sources on digestibility of protein.

The effect of protein levels and energy sources on ileal protein digestibility was shown in Table 4.7 and 4.8. There were no interactions between protein levels and energy sources on ileal protein digestibility in both 21 and 42 days of age. On day 21 both dietary protein levels and energy sources did not influence ileal protein digestibility.

On day 42, dietary protein levels affected digestibility of protein. Birds fed on 85% protein diet had lowest protein digestibility ($P<0.05$). The sources of energy also influenced ileal protein digestibility. Birds fed CC diet had significantly lower protein digestibility ($P<0.0001$) than those birds fed CO diet. Birds fed 95% protein in CO diet had highest protein digestible coefficient (0.79) that was significantly different from those birds fed 85 and 90% protein in CC diets (0.69) but not significantly different from bird in other remaining groups.

4.4 Effect of protein levels and energy sources on excretion of nitrogen in the feces.

The effect of protein levels and energy sources on excretion of nitrogen in the feces was shown in Table 4.7 and 4.8. There was no interaction between protein levels and energy sources on nitrogen excretion in the feces in both phases. However, dietary protein levels affected fecal nitrogen which was decreased as dietary protein declined in both grower ($P<0.001$) and finisher ($P<0.05$) periods. Birds fed 85% protein in CO diet had lowest fecal nitrogen (23.7, 28.2 g/kg) whereas birds fed 100% protein in CC diet had highest fecal nitrogen (28.0, 33.5 g/kg) in grower and finisher periods, respectively.

Table 4.7 Effect of protein levels and energy sources on ileal protein digestibility and excretion of nitrogen in the feces on day 21

Treatment			Ileal protein digestibility	N-excretion
Group	Energy source	Protein level (% Cobb Std.)	(Coeff.)	(g/kg)
1	CO	100	0.85	26.9[ab]
2	CO	95	0.85	26.0[abc]
3	CO	90	0.86	24.1[c]
4	CO	85	0.84	23.7[c]
5	CC	100	0.83	28.0[a]
6	CC	95	0.84	27.2[ab]
7	CC	90	0.84	25.4[bc]
8	CC	85	0.84	24.3[c]
SEM			0.31	0.34
CV, %			2.57	7.75
Source			*P-value*	
	Treatment diet		0.5615	0.0034
	Protein levels (A)		0.6356	0.0005
	Energy sources (B)		0.1441	0.0669
	(A) x (B)		0.5900	0.9691
Main effect				
Protein level				
	100%		0.84	27.4[a]
	95%		0.84	26.6[a]
	90%		0.85	24.8[b]
	85%		0.84	24.0[b]
Energy source				
	CO		84.8	25.2
	CC		83.9	26.2

*[a,b,c] Means in a column with different superscripts are significantly different (P<0.05)

Table 4.8 Effect of protein levels and energy sources on ileal protein digestibility and excretion of nitrogen in the feces on day 42

Treatment			Ileal protein digestibility	N-excretion
Group	Energy source	Protein level (% Cobb Std.)	(Coeff.)	(g/kg)
1	CO	100	0.78[a]	32.3[ab]
2	CO	95	0.79[a]	32.1[ab]
3	CO	90	0.75[ab]	29.8[bcd]
4	CO	85	0.75[ab]	28.2[d]
5	CC	100	0.75[ab]	33.5[a]
6	CC	95	0.71[bc]	31.7[abc]
7	CC	90	0.69[c]	29.8[bcd]
8	CC	85	0.69[c]	28.7[cd]
SEM			0.75	0.46
CV, %			5.67	9.18
Source			*P-value*	
	Treatment diet		0.0009	0.0206
	Protein levels (A)		0.0299	0.0016
	Energy sources (B)		<0.0001	0.7055
	(A) x (B)		0.4816	0.9020
Main effect				
Protein level				
	100%		0.76[a]	32.9[a]
	95%		0.75[ab]	31.9[ab]
	90%		0.72[b]	29.8[bc]
	85%		0.72[b]	28.5[c]
Energy source				
	CO		0.77[a]	30.6
	CC		0.71[b]	30.9

*[a,b,c,d] Means in a column with different superscripts are significantly different (P<0.05)

CHAPTER V
DISCUSSION

The analyzed results of HCN in cassava pellets composed in grower and finisher diet were less than 5 and 6.8 mg/kg which were lower than the toxic levels at 22.97 and 50 mg/kg recommended by Khajarern et al. (1982) and Garcia (1999), respectively. Therefore, the effect of HCN on growth performance should be dismissed.

There were some analyzed AA values that slightly less than calculation (Table 4.1 and 4.2). Total sulfur amino acids (Met + Cys) were the most concern because Met had been considered as the first limiting AA (Ojano-Dirain and Waldroup, 2002). In current study, analyzed M+C values were slightly lower than calculated values in both grower (0.75-0.80 vs 0.84%) and finisher diets (0.78-0.81 vs 0.82%) compared with Cobb (2008) recommendation. However, the ranks of analyzed M+C levels in this study were higher than the levels recommended by Aftab and Ashraf (2009) who reported that M+C requirements for Hubbard broilers during 1-21 and 22-42 days of age were 0.75 and 0.67%, respectively. Moreover, the minimum analyzed Met values in the present study were also higher than the values suggested by Chamruspollert et al. (2004) in Ross 208 broilers and Ojano-Dirain and Waldroup (2002) in male Cobb 500 broilers for grower (0.46 vs 0.43%) and finisher (0.49 vs 0.44%) periods, respectively. Lys values were slightly lower than requirements in 100%CO and 100, 95%CC diets (1.04, 1.02, 1.02 vs 1.05%) but these values were higher than 0.99% which reported by Labadan et al. (2001) in male broilers (Ross male x Avian female). The minimum analyzed results of Thr and Trp in all diets were 0.68, 0.18% in grower and 0.67, 0.17% in finisher periods which were also lower than requirements. Nonetheless, the previous studies showed the requirements of Thr and Trp were 0.68 (Smith and Waldroup, 1988a), 0.16% (Smith and Waldroup, 1988b) in male (Vantress x Arbor Acres) broilers in grower and 0.63 in male (Ross 208) broilers (Mack et al., 1999), 0.17% in male and female (Cobb) broilers (Freeman, 1979) in finisher periods, respectively. Therefore, the consequence of the slightly lower in some amino acid values should have no any effect on growth performance.

5.1 Effect of protein levels and energy sources on growth performance

During 1-21 days of age (Table 4.3), reducing dietary protein levels up to 85% of requirement did not affect feed intake, ADG, and final body weight. However, FCR in 85% protein group was significantly higher compared with the other protein levels ($P<0.05$). This was in accordance with the study of Ferguson et al. (1998a, b) who found that reducing protein from 22.0 to 20.0% and 26.4 to 21.9% in the isocaloric diets (3,200 and 3,100 kcal/kg ME) supplemented with synthetic amino acids to meet requirement during starter period (1-21d) did not adversely affect feed intake and ADG but increased FCR. The same unalterable result of feed consumption was also found by Dean et al. (2006) who decreased protein in the isocaloric diets (3,200 kcal/kg ME) from 22.18 to 16.18% with supplementation of synthetic AA during 1-21 days of age.

During 22-42 days of age, feed intake was not affected by protein levels but significant difference was found in energy sources and interaction between energy sources and protein levels on feed intake in both finisher and overall periods ($P<0.05$). The interaction during finisher period (Table 4.4) showed that feed intake was lowest in 85% protein in CO diet (2,894.9 g/b) and highest in 90%CC diet. The potential cause of this interaction was final weight of 85% protein in CO diet which was lowest in grower period even though it was not significant difference leading to decrease feed consumption in finishing period compared with other groups. Moreover, analyzed gross energy (GE) in all CO diets were higher than all CC diets inducing to higher feed consumption of birds fed CC diets compared with CO diets. An additional probable factor may be an addition of one or more amino acids to low protein diets eventually limited appetite and feed intake leading to poor growth rate (Lee, 2009). According to the study of Waguespack et al. (2009) who added L-Lys·HCl at 0.05% increments from 0.25 to 0.60% in the decreased protein broiler diets from 22.2 to 17.4% with isoenergetic (3,200 kcal/kg ME) during 0-18 days of age. They found that supplementation of L-Lys·HCl at 0.30% started to reduce feed intake. The similar result was found by Namroud et al. (2008) who decreased dietary protein from 23 to 17% protein by

supplementing of L-Lys·HCl from 0 to 0.37%, respectively, in isocaloric (3,175 kcal/kg ME) broiler diets during 0-28 days of age. The result showed that adding 0.37% L-Lys·HCl to 17% protein diet significantly reduced feed intake ($P<0.05$). This phenomenon on decreasing feed intake in current study had a trend in first phase (Table 4.3) and clearly shown in second phase (Table 4.4) of 85% protein in CO diet which was supplemented L-Lys·HCl at the highest level of 0.39 and 0.40% (Table 3.1 and 3.2). Feed consumption was not affected by protein levels in finisher and overall periods that in agreement with previous studies (Bartov and Plavnik, 1998; Kamran et al., 2004).

The effect of protein levels showed the negative effect on growth performance when reducing dietary protein up to 85% of requirement, whereas final body weight, ADG, and FCR of chicks fed 90% protein group did not differ from chicks fed 100% protein group. This results were similar with other previous studies which concluded that dietary protein could be reduced by 2 percentage units (Khajali and Moghaddam, 2006) and should not more than three percentage units (Ferguson et al., 1998a; Aletor et al., 2000).

An additional possible factor in lowering growth performance when decreasing dietary protein may be insufficient nonessential AAs (NEAA). In this study, we mainly concentrated only on 4 EAA but some NEAA are also important to support growth performance. It had been reported that NEAA were necessary for optimum bird performance and body composition due to the synthesis of NEAA was a limiting factor in low protein diets (Han et al., 1992). Dean et al, (2006) indicated the importance of NEAA. They suggested that in low protein diet (16.21%), supplementation of synthetic EAA alone could not compensate growth performance to a level equal to those of birds fed control diet (22.21%) but when NEAA were also added, feed efficiency of low protein chicks increased equally to the level of control chicks. Furthermore, the previous study found that even if EAA met or exceeded the minimum requirement, insufficiency of some NEAA could not support for maximum protein synthesis and eventually restrict growth

(Fancher and Jensen, 1989). Corzo et al. (2005) demonstrated that reducing protein in the grower diet (1-21d) from 22.0 to 18.0% should add NEAA to improve growth performance equal to control group. In current study, even though almost NEAA levels in diets used met or above the requirement, most of them tend to decrease as dietary protein levels decline. It might cause in inferior growth performance especially in lower protein diets which had higher gap compared with the requirement.

A possible factor that affected growth performance when reducing dietary protein levels may be AA profiles relative to Lys. In grower period of this study, the requirement M+C levels relative to Lys were 0.82 and 0.76 recommended by NRC (1994) and Cobb-Vantress (2008), respectively. While the previous study showed that M+C requirement relative to Lys was 0.62 (Aftab and Ashraf, 2009) in grower period which was lower than the minimum values at 0.69 in this study. The minimum M+C level in finisher period was 0.73 which was higher than 0.72 that recommended by NRC (1994) but slightly lower than Cobb-Vantress (2008) recommendation (0.78). The minimum Thr levels relative to Lys in grower and finisher period of current study were 0.63 and 0.64%, while the recommendation levels were 0.73, 0.74 (NRC, 1994) and 0.67, 0.69 (Cobb-Vantress, 2008), respectively. Nonetheless, the previous studies showed that the Thr requirement relative to Lys were 0.63 (Mack et al., 1999) and 0.56% (Baker et al., 2002) in grower and finisher periods, respectively. The levels of Trp relative to Lys in grower period were in agreement with the recommendation levels suggested by NRC (1994) and Cobb-Vantress (2008) and the lowest relative ration of Trp to Lys in finisher period was 0.16 which was very closed to 0.18 recommended by both NRC (1994) and Cobb-Vantress (2008). Although the main limiting AA (M+C, Thr and Trp) levels relative to Lys were in the range of previous suggestion, there were some EAA such as Val, Ile, and Arg which were lower ratios relative to Lys (compared with NRC (1994) recommendation) in some treatments especially when dietary protein levels were reduced to 90 and 85% of recommendation. The insufficiency of some EAA might induce to lower performance in 90 and 85% CP diets compared with 100 and 95%CP diets.

There were also evidence that birds fed CC diets consumed more feed than birds fed CO diets in both finisher ($P<0.01$) and overall ($P<0.01$) periods (Table 4.4 and 4.5) and eventually resulted in significantly higher FCR ($P<0.01$) in overall period. In this study, dietary fiber in CC diets were slightly higher than CO diets in both grower (3.48-3.73 vs 2.57-2.85) and finisher (3.41-3.97 vs 2.55-2.69) periods (Table 4.1 and 4.2). However, the previous studies showed that dietary fiber varying from 2.5 to 3.4% did not affect feed intake in Cobb 500 broilers during 1-21 days of age (Gonzalez-Alvarado et al., (2007) and the dietary fiber level varying from 1.30-3.76% did not alter feed intake in Hy –Line W36 laying hen during 23-58 weeks of age (Roberts et al., 2007). Furthermore, Bhatti et al, (2002) investigated the fiber content in commercial poultry diets and found that the fiber levels varied from 4.79 to 5.69%. For fat content, CO diets were higher than CC diets in both periods (Table 4.1 and 4.2). Jafarnejad and Sadegh (2011) demonstrated that no significant differences in body weight and FCR during 1-21 days of age in male Ross 308 fed diets contained calculated fat varied from 5.1 to 10.0%. Moreover, Rosebrough et al, (1999) reported that feeding the diets contained 3, 6 and 13% calculated fat in male Indian River broiler chicks during 7-28 days of age did not influence on feed intake and feed conversion efficiency. Thus the effect of fiber and fat levels on growth performance in current study should be dismissed.

The higher feed consumption of birds fed CC diet in both periods resulted in higher feed intake in overall period (Table 4.5). It had been reported that cassava had faster rate of passage (Weurding et al., 2001) because of higher branch chain of amylopectin and more amount of rapidly digestible starch (14.83 vs 6.25% DM). Furthermore, 70% of cassava content in the diets was digested at upper digestive tract (Promthong et al., 2006) thus some energy was used to supply or maintain epithelial cell of GI tract supported by Cant et al. (1996). They explained that the gastrointestinal (GI) tract used about 20% of all dietary energy to maintain digestive and absorptive processes. In addition, Fleming et al, (1997) indicated that glucose and glutamine were primarily used to provide energy for the small intestine (SI). Therefore, the diet that contained starch from cassava was digested in the upper SI could not able to supply

glucose for itself activity in the lower part of SI leading to more AA may be oxidized for SI activity. From the mentioned reasons, it can be implied that birds fed CC diets should be had lower ADG than birds fed CO diets but the result showed that no difference in ADG (75.41 vs 73.20 g/b/d) because birds fed CC diets consumed more feed (3,200.5 vs 3,021.5 g/b) than birds fed CO diets.

5.2 Effect of protein levels and energy sources on economic returns

In terms of economic returns, the price of ingredients used for calculation based on the day that diets were mixed, reducing dietary protein at 85% of requirement showed unsatisfactory values in all parameters. However all of the economic parameters at 90% protein group were not significantly different from 100 and 95% protein groups. Substitution of cassava for corn by 50% had no adverse effect on economic values in all protein levels. Birds fed 100%CO diet had the highest EEF (372.9), whereas birds fed 95%CC diet had the lowest feed cost/kg of live weight (24.3 ฿/kg). Moye (2003) reported that EEF and feed cost/kg of live weight had a direct inverse relationship. The birds had higher growth rate and lower FCR normally showed lower cost. Besides, broiler live cost also depended on grain and other ingredient prices. In this study, birds in 95%CC group had insignificant different ADG, FCR, and feed intake compared with birds in 100%CO group (Table 5) but they ate the cheaper feed in both grower (12.05 vs 12.66 ฿/kg) and finisher (12.22 vs 12.75 ฿/kg) periods (Table 3.1 and 3.2) leading to have the lowest live cost and the highest income/m^2 (500.6 ฿/ m^2). The minimum accepted value for broiler production of EEF was 260 and the higher value was better (Abbas et al., 2006). Zakeri and Kashefi, (2011) studied the effects of five growth promoters on Cobb 500 performance. The results showed that EEF varied from 308 to 349. These values were closed to the values in this study that EEF varied from 284.8 in CO85% group to 372.9 in CO100% group which was higher than both mentioned above and also low mortality rate (<3%), it can imply that quality of diets and management system in current trial are in the higher standard level. Nevertheless, the price of CC diet might not cheaper than CO diet in all situations depending on the price of feed ingredients

especially soybean meal (SBM). In this study, when corn was replaced with cassava, SBM was added around 4% in all decreased protein levels in both periods (Table 3.1 and 3.2). Therefore, if the price of SBM increases, the price of CC diet may be higher than CO diet along with the slightly lower growth performance and can eventually lead to undesirable economic values. In conclusion, under this condition reducing protein in the diet up to 90% of requirement gave the same economic return as normal protein levels.

5.3 Effect of protein levels and energy sources on ileal protein digestibility

During grower period (1-21d), dietary protein as well as energy sources did not affect ileal protein digestibility (Table 4.7). The same trend of protein digestibility was also found in the study of Stringhini et al. (2009) who fed the MPK male broilers with different protein levels diets varying from 20 to 24% by supplementing of synthetic AA during 1-7 days of age. The result showed insignificant protein digestibility among groups which varied from 57.3 to 60.6%.

In general, when dietary protein was insufficient for the requirement, birds should utilize the diet efficiently so the digestibility coefficient of lower protein ration should be higher. In contrast with the current research, protein digestibility was decreased with reducing of dietary protein despite almost EAA were fortified with synthetic AA which had been accepted as high digestibility nutrients. This scenario may be explained by a concept of protein absorption. D'Mello (2003) explained that digested AA in luminal phase would be absorbed in both form of free AA and small peptides. However, about 70-85% of all luminal AA were absorbed into enterocytes in the form of small peptides and the rate of absorption was faster compared with absorption in free AA form. This concept was in agreement with Bregendahl et al, (2002) mentioned that dietary free AA may be metabolized in the enterocytes, decreasing their bioavailability compared to that of AA absorbed in peptide form. Furthermore, Mavromichalis and Baker, (2000) concluded that the bioavailability of free AA in low protein diets may

decline because of Maillard reactions after only one week of storage under warm and humid conditions. In addition, the levels of adding synthetic AA in current study were higher in the lower protein diets resulting in the significantly lowest ($P<0.001$) protein digestibility coefficient in 85% protein diet compared with other protein groups. This phenomenon can be explained by the reason mentioned above and also collaborated with the results of many previous studies of low protein diets supplemented with crystalline AA (Short et al., 1999; Kamisoyama et al., 2009).

When considered the difference in nutritional content (fat and fiber) among experimental diets, there was reported that no effect on ileal protein digestibility was found in male Single Comb White Leghorn chicks fed different dietary fat contents varying from 3.0 to 10.0% (Honda et al., 2009) but not for fiber. It had been indicated a strong negative correlation between crude fiber content in the diet and protein as well as fat digestibility but the low crude fiber diet improved broiler performance as cited by Jimenez-Moreno et al., (2009). Due to CC had high fiber content than CO thus protein digestibility was lower. Another possible reason could be explained by non-starch polysaccharide (NSP) which reduced the utilization of protein in the diets as reported by Fuente et al. (1998). They explained that NSP reduced protein digestibility by increasing intestinal viscosity and reducing contact of enzyme and substrate. It had been reported that the NSP content in cassava pellet had significantly higher (14.08 vs 9.23% DM) than corn grain (Promthong et al., 2005).

5.4 Effect of protein levels and energy sources on excretion of nitrogen in the feces

Nitrogen excretion decreased when dietary protein declined in 90 and 85% compared with 100 and 95% protein groups ($P<0.001$) in grower period and lower in 85% compared with 95 and 100% protein groups ($P<0.05$) in finisher period (Table 4.7 and 4.8). These results corresponded with previous study that fecal nitrogen declined with decreasing dietary protein (Furguson et al., 1998a; Khajali and Moghaddam, 2006). The reduction of nitrogen might be explained by the reduction of protein intake as

reducing dietary protein. According the calculation of protein intake in all diets, it decreased from 247.3 to 208.3g/b in grower and from 553.8 to 481.1g/b in finisher periods. In addition, the ratio of EAA relative to Lys in the lower CP diets had lower than the higher CP diets. From both reasons resulted to lower nitrogen excreted in the feces in the lower CP diets compared with the higher CP diets.

In current study for every percentage point reduction in dietary CP, the nitrogen contents in the feces were decreased 4.60 and 5.35% at 21 and 42 days of age, respectively. The percentage of nitrogen reduction per unit of reducing dietary CP in this study was in accordance with the previous studies. Namroud et al. (2008) reported that when dietary CP was decreased from 23.12 to 17.0%, fecal nitrogen declined 4.50% per unit of dietary CP reduction in male (Ross 308) broilers during 1-21 days of age. Furthermore, Bregendahl et al. (2002) found that decreasing dietary CP from 24.00 to 18.50%, nitrogen excretion in the feces decreased 5.57% per unit of dietary CP reduction in male (Hubbard) broilers during 1-21 days of age. In addition, Yamazaki et al. (2007) pointed out that decreasing dietary CP from 17.0 to 15.0% during 22-42 days of age in male (Chunky) broilers, fecal nitrogen decreased around 5.0% per unit of dietary CP reduction.

Energy sources did not affected nitrogen excretion in the feces in both grower and finisher periods. According to CC diets had higher NSP than CO diets, leading to lower digestibility that mentioned in 5.3 and slightly higher fecal nitrogen in both periods (Table 4.7 and 4.8).

In conclusion, dietary crude protein can be reduced up to 95% of recommendation by supplementing of the fourth primary limiting synthetic EAA with maintained energy levels and cassava can be used to substitute for corn by 50% when considered only growth performance and economic returns. If environmental pollution was included in the objective, the crude protein should be decreased to 90% of recommendation.

REFERENCES

Abbas, T., Muneer, M. A., Ahmed, M. D., Khan, M. A., Younus, M. and Khan, I. 2006. Comparative efficacy of five different brands of commercial Newcastle disease lasota virus vaccines in broilers. Pakinstan Vet. J. 26(2): 55-58.

Aftab, U. and Ashraf, M. 2009. Methionine+cystine requirement of broiler chickens fed low-density diets under tropical conditions. Trop. Anim. Healt. Prod. 41: 363-369.

Aletor V. A., Hamid II., Nieb E. and Pfeffer E. 2000. Low-protein amino acid-supplemented diets in broiler chickens: effects on performance, carcass characteristics, whole-body composition and efficiencies of nutrient utilization. J. Sci. Food. Agric. 80: 547-554.

Association of Official Analytical Chemists. 1990. Official Methods of Analysis. 15th ed. Arlington, VA., USA.

Association of Official Analytical Chemists. 1995. Official Methods of Analysis. 16th ed. Arlington, VA, USA.

Association of Official Analytical Chemists International. 2006. Official Methods of Analysis. 18th ed. Gaithersburg, MD, USA.

Ayasan, T., Okan, F. and Hizli, H. 2009. Threonine requirement of broiler from 22-42 days. Int. J. Poult. Sci. 8(9): 862-865.

Baker, D. H., Batal, A. B., Parr, T. M., Augspurger, N. R. and Parsons, C. M. 2002. Ideal ratio (relative to lysine) of tryptophan, threonine, isoleucine, and valine for chicks during the second and third weeks posthatch. Poult. Sci. 81: 485-494.

Bartov, I. and Plavnik, I. 1998. Moderate excess of dietary protein increases breast meat yield of broiler chicks. Poult. Sci. 77: 680-688.

Bhatti, B. M., Talat, T. and Sardar, R. 2002. Estimation of crude fiber and crude protein in commercial poultry rations and some important feed ingredients. Pakistan Vet. J. 22(1): 5-7.

Bregendahl, K., Sell., J. L. and Zimmerman, D. R. 2002. Effect of low-protein diets on growth performance and body composition of broiler chicks. Poult. Sci. 81: 1156-1167.

Cant, J. P., McBride, B. W. and Jr., Croom, Jr. W. J. 1996. The regulation of intestinal metabolism and its impact on whole animal energetic. J. Anim. Sci. 74: 2541-2553.

Chamruspollert, M., Pesti, G. M. and Bakalli, R. I. 2004. Influence of temperature on the arginine and methionine requirements of young broiler chicks. J. Appl. Poult. Res. 13: 628-638.

Chauynarong, N., Elangovan, A. V. and Iji, P.A. 2009. The potential of cassava products in diet for poultry. World's Poult. Sci. J. 65: 23-36.

Cobb Vantress Inc. 2008. "Cobb Broiler Performance and Nutrition Supplement, Rev. Europe, Middle East, Africa version." [Online]. Available:http://www.cobb-vantress.com

Corzo A., Fritts C.A., Kidd M.T. and Kerr B.J. 2005. Response of broiler chicks to essential and non-essential amino acid supplementation of low crude protein diets. Anim. Feed. Sci. 118:319-327.

Dean, D. W., Bidner, T. D. and Southern, L. L. 2006. Glycine supplementation to low protein amino acid-supplemented diets supports optimal performance of broiler chicks. Poult. Sci. 85: 288-296.

D'Mello, J .P. F. 2003. An outline of pathways in amino acid metabolism. In: Amino Acids in Animal Nutrition 2nd ed. (D'Mello, J.P.F., ed.) pp:71-86. CABI International, Oxon, UK.

Fancher, B. I. and Jensen, L. S. 1989. Dietary protein levels and essential amino acid content: influence upon female broiler performance during the grower period. Poult. Sci. 68: 897-908.

Ferguson, N. S., Gates, R. S., Taraba, J. L., Cantor, A. J., Pescatore, A. J., Straw, M. L., Ford, M. J. and Burnhams, D. J. 1998a. The effect of dietary protein and phosphorus on ammonia concentration and litter composition in broilers. Poult. Sci. 77: 1085-1093.

Ferguson, N. S., Gates, R. S., Taraba, J. L., Cantor, A. J., Pescatore, Ford, M.J. and Burnham, D. J. 1998b. The effect of dietary crude protein on growth, ammonia concentration, and litter composition in broilers. Poult. Sci. 77: 1481-1487.

Ferket, P. R., Heugten, E. van., Kempen, T. A. T. G. van. and Angel, R. 2002. Nutrition strategies to reduce environmental emission from nonruminants. J. Anim. Sci. 80(E. Suppl. 2): E168-E182.

Fleming, S. E., Zambell, K. L. and Fitch, M. D. 1997. Glucose and glutamine provide similar proportions of energy to mucosal cells of rat small intestine. Am. J. Physiol. 273: G968-978.

Freeman, C.P. 1979. The tryptophan requirement of broiler chicks. Br. Poult. Sci. 20: 27-37.

Fuente, J. M., Ayala, P. D., Flores, A. and Villamide, M. J. 1998. Effect of storage time and dietary enzyme on the metabolizable energy and digesta viscosity of barley-based diets for poultry. Poult. Sci. 77: 90-97.

Fuller, M. F. 2004. The encyclopedia of farm animal nutrition. Aberdeen : CABI. 18-447.

Garcia, M. 1999. Cassava root meal for poultry. J. Appl. Poult. Res. 8: 132-137.

Gonzalez-Alvarado, J. M., Jimenez-Moreno, E., Lazaro, R. and Matoes, G. G. 2007. Effect of type of cereal, heat processing of the cereal, and inclusion of fiber in the diet on productive performance and digestive traits of broilers. Poult. Sci. 1705-1715.

Han, Y., Suzuki, H., Parsons, C. M. and Baker, D. H. 1992. Amino acid fortification of a low-protein corn and soybean meal diet for chicks. Poult. Sci.71: 1168-1178.

Honda, K., Kamisoyama, H., Isshiki, Y. and Hasegawa, S. 2009. Effects of dietary fat levels on nutrient digestibility at different sites of chicken intestines. Japan. Poult. Sci. 46: 291-295.

Jafarnejad, S. and Sadegh, M. 2011. The effects of different levels of dietary protein, energy and using fat on the performance of broiler chicks at the end of the third weeks. Asian J. Poult. Sci. 5: 35-40.

Jimenez-Moreno, E., Gonzalez-Alvarado, J. M., Lazaro, R. and Mateos, G. G. 2009. Effects of type of cereal, heat processing of the cereal, and fiber inclusion in the diet on gizzard pH and nutrient utilization in broilers at different ages. Poult. Sci. 88: 1925-1933.

Kamisoyama, H., Honda, K., Isshiki, Y. and Hasegawa, S. 2009. Effects of dietary protein levels on the nutrient digestibility at different sites of chicken intestines. Japan. Poult. Sci. 46: 193-197.

Kamran, Z., Aslam, M. M., Ahsan-ul-Haq. and Mahmood, S. 2004. Effect of decreasing dietary protein levels with optimal amino acid profile on the performance of broilers. Pakistan Vet. J. 24(4): 165-168.

Kamran, Z., Sarwar, M., Nisa, M., Nadeem, M. A., Mahmood, S., Babar, M. E. and Ahmed, S. 2008.Effect of low-protein diets having constant energy-to-protein ratio on performance and carcass characteristics of broiler chickens from one to thirty-five days of age. Poult. Sci. 87: 468-474.

Kanto, U., Juttupornpong, S. 2004. Using cassava as animal feeds. Thai tapioca development institute: Bangkok. 10-13.

Khajali, F. and Moghaddam, H. N. 2006. Methionine supplementation of low-protein broiler diets: influence upon growth performance and efficiency of protein utilization. Int. J. Poult. Sci. 5(6): 569-573.

Khajarern, J., Khajarern, S., Sivapraphagon, A. and Nandhapipat, L. 1982. A survey on the changes in chemical composition of cassava root products in Khon Kaen region in 1980. In KKU-IDRC Cassava/ Nutrition project 1976 Annual Report, Khon Kaen University, Khon Kaen, Thailand: 22-29.

Kidd, M. T. and Kerr, B. J. 1996. L-threonine for poultry: a review. J. Appl. Poult. Res. 5: 358-367.

Kim, W. K., Froelich, C. A., Froelich, Patterson, P. H. and Ricke, S.C. 2006. The potential to reduce poultry nitrogen emissions with dietary methionine or methionine analogues supplementation. World's Poult. Sci. J. 62: 338-353.

Labadan, M. C. Jr., Hsu, K. N. and Austic, R. E. 2001. Lysine and arginine requirements of broiler chickens at two to three-week intervals to eight weeks of age. Poult. Sci. 80: 599-606.

Lee, I. Chiba. 2009. "Animal nutrition handbook" [Online]. Available: http://bibliotecamvz.blogspot.com/2010_05_01_archive.html

Leeson, S. and Summers, J. 2001. Proteins and amino acids. In : Nutrition of the chicken. 4th (ed). Ontario : Ithaca. 102-110.

Mack, S., Bercovici, D., Groote, G. D., Leclercq, B. Lippens, M., Pack, M., Schutte, J. B. and Cauwenberghe, S. V. 1999. Ideal amino acid profile and dietary lysine specification for broiler chickens of 20 to 40 days of age. Br. Poult. Sci. 40: 257-265.

Mavromichalis, I. and Baker, D. H. 2000. Effects of pelleting and storage of a complex nursery pig diet on lysine bioavailability. J. Anim. Sci. 78: 341-347.

Moye, J. 2003. “Making the breed decision”. [Online]. Available: http://www.cobb-vantress.com/Publications/documents/focus_product_1-03.pdf

Nahm, K. H. 2007. Feed formulations to reduce N excretion and ammonia emission from poultry manure. Bio. Tech. 98: 2282-2300.

Namroud, N. F., Shivazad, M. and Zaghari, M. 2008. Effects of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks. Poult. Sci. 87: 2250-2258.

National Research Council. 1994. Nutrient Requirements of Poultry: 9th Rev. ed. National Academy Press, Washington, DC.

Ndegwa, P. M., Hristov, A. N., Arogo, J. and Sheffield, R. E. 2008. A review of ammonia emission mitigation techniques for concentrated animal feeding operations. Bio. Eng. 100: 453-469.

Office of Agricultural Economics. 2010. “Agricultural statistics” [Online]. Available: http://www.oae.go.th/main.php?filename=index

Official Journal of the European Communities. 1998. “Commission directive 98/64/ec”. [Online]. Available: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:1998:257:0014:0028:EN:PDF

Ojano-Dirain, C. P. and Waldroup, P. W. 2002. Evaluation of lysine, methionine and threonine needs of broilers three to six week of age under moderate temperature stress. Int. J. Poult. Sci. 1(1): 16-21.

Oviedo-Rondon, E. O., Clemente-Hernandez, S., Salvador-Torres, F. and Stephen, F. 2005. “Effect of low crude protein diets on broiler performance, breast meat and carcass yield”. [Online]. Available:http://www.lysine.com/new/Technical%20Reports/Poultry/PRR25.pdf

Perry, T. W., Arthur E. and Robert S. 1999. Feeds and feeding. In : Protein nutrition. 5th (ed). New Jersey : Prentice-Hall, Inc. 71-73.

Promthong, S., Kanto, U., Tirawattanawanich, C., Tongyai, S., Isariyodom, S., Markcichitr, K.. and Engkagul, A. 2005. Comparison of nutrient compositions and carbohydrate fractions of corn, cassava chip and cassava pellet ingredients: Animals. Proceedings of 43rd Kasetsart University Annual Conference, Thailand.

Promthong, S., Kanto, U., Tirawattanawanich, C., Tongyai, S., Isariyodom, S., Markcichitr, K. and Engkagul, A. 2006. Comparative effects of corn, cassava chip and cassava pellet diets on histological properties in broiler intestinal tract: Animals Veterinary Medicine. Proceedings of 44th Kasetsart University Annual Conference, Thailand. 211-220.

Rezaei, M., Nassir, M. H., Pour, R. J. and Kermanshahi, H. 2004. The effects of dietary protein and lysine levels on broiler performance, carcass characteristics and n excretion. Int. J. Poult. Sci. 3(2): 148-152.

Roberts, S. A., Xin, H., Kerr, B. J., Russell, J. R. and Bregendahl, K. 2007. Effects of dietary fiber and reduced crude protein on nitrogen balance and egg production in laying hens. Poult. Sci. 86: 1716-1725.

Rosa, A. P., Pesti, G. M., Edwards, H. M. Edwards, Jr. and Bakalli, R. 2001. Tryptophan requirements of different broiler genotypes. Poult. Sci. 80: 1718-1722.

Rosebrough, R. W., Mcmurtry, J. P. and Vasilatos-yunken, R. 1999. Dietary fat and protein interactions in broiler. Poult. Sci. 78: 992-998.

Saentaweesuk, S., Kanto, U., Juttupornpong, S. and Harinsut, P. 2000. Substitution of cassava for corn in broiler diets. In : The Proceeding of 38th Kasetsart University Annual Conference. Bangkok, Thailand, February 1-4: 236.

Samadi and Frank, L. 2007. Lysine requirement of fast growing chickens – effects of age, sex, level of protein deposition and dietary lysine efficiency. Japan. Poult. Sci. 44: 63-72.

Short, F. J., Wiseman, J. and Boorman, K. N. 1999. Application of a method to determine ileal digestibility in broilers of amino acids in wheat. Anim. Feed Sci. Technol. 79: 195-209.

Smith N. K. and Waldroup, P. W. 1988a. Investigations of threonine requirements of broiler chicks fed diets based on grain sorghum and soybean meal. Poult. Sci. 67: 108-112.

Smith N. K. and Waldroup, P. W. 1988b. Estimation of the tryptophan requirement of male broiler chickens. Poult. Sci. 67: 1174-1177.

Steenfeldt, S., Gonzalez, E. and Bach, K. E. 2003. Effects of inclusion with blue lupins (Lupinus angustifolius) in broiler diets and enzyme supplementation on production performance, digestibility and dietary AME content. Anim. Feed Sci. Techno. 110: 185-200.

Stringhini, J. H., Andrade, M. L., Xavier, S. A. G., Andrade, M. A., Minafra, C. S. and Café, M. B. 2009. Protein and amino acid supplementation levels for broilers in pre-starter ration. Int. J. Poult. Sci. 8(10): 946-951.

Suvanatad, C. 2003. Effect of turmeric (*curcuma longa linn*.) as an antioxidant on immune status and growth performances of stressed broilers. (dissertation). Chulalongkorn University. 71p.

Todd, J. and Angel, R. 2008. “Protien and amino acid requirements for poultry”. [Online]. Available: http://www.ces.purdue.edu/extmedia/AS/AS-584-W.pdf

Waguespack, A. M., Powell, S., Bidner, T. D., Payne, R. L. and Southern, L. L. 2009. Effect of incremental levels of L-lysine and determination of the limiting amino acids in low crude protein corn-soybean meal diets for broilers. Poult. Sci. 88: 1216-1226.

Weurding, R. E., Veldman, A., Veen W. A. G., Van der Aar, J. P. and Verstegen, M. W. A. 2001. Starch digestion rate in the small intestine of broiler chickens differs among feedstuffs. J. Nutr. 131: 2329-2335.

Williams, C.H. and David, D.J. 1962. The determination of chromic oxide in feces sample by atomic absortion spectrophotometry. J. Agric. Sci. 59:381-385.

Yamazaki, M., Murakami, H., Nakashima, K., Otsuka, M., Takada, R. and Abe, H. 2007. Effect of cellulose supplementation in low-crude protein diets on performance, nitrogen excretion, fat deposition, hepatic lipogenic and lipolytic enzyme activity in broilers. Br. Poult. Sci. 48(2): 211-216.

Zaghari, M., Zaefarian, F. and Shivazad, M. 2011. Standardized ileal digestibility threonine requirements and its effects on performance and gut morphology of broiler chicks fed two levels of protein. J. Agric. Sci. Tech. 13: 541-552.

Zakeri, A. and Kashefi, P. 2011. The comparative effects of five growth promoters on broiler chickens humoral immunity and performance. J. Anim. Vet. Adv. 10(9): 1097-1101.

Zhao, J. P., Chen J. L., Zhao, G. P., Zheng, M. Q., Jiang, R. R. and Wen, J. 2009. Live performance, carcass composition, and blood metabolite responses to dietary nutrient density in two distinct broiler breeds of male chickens. Poult. Sci. 88: 2575-2584.

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