DIGESTIBLE LYSINE AND SULFUR AMINO ACID REQUIREMENTS OF KORAT CHICKENS

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Animal Production Technology

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ความต้องการกรดอะมิโนไลซีนและซัลเฟอร์ที่ย่อยได้ของไก่โคราช

นางทราน ฮอง ดินห์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาปรัชญาดุษฎีบัณฑิต
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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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ทราน ฮอง ดินห์ : ความต้องการกรดอะมิโนไลซีนและซัลเฟอร์ที่ย่อยได้ของไก่โคราช (DIGESTIBLE LYSINE AND SULFUR AMINO ACID REQUIREMENTS OF KORAT CHICKENS) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร. สุทิศา เข็มผะกา, 125 หน้า.

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาความต้องการกรดอะมิโนไลซีนและซัลเฟอร์ของไก่โคราช ในช่วงอายุ 0-12 สัปดาห์ โดยแบ่งออกเป็น 2 การทดลอง

การทดลองที่ 1 เป็นการศึกษาความต้องการไลซีนของไก่โคราชช่วงอายุ 0-12 สัปดาห์ แบ่งออกเป็น 4 ระยะการทดลองตามช่วงอายุ 0-3 3-6 6-9 และ 9-12 สัปดาห์ ในแต่ละระยะการทดลองกำหนดให้มีไลซีน 5 ระดับ คือ 0.87 0.97 1.07 1.17 และ 1.27% ในช่วงอายุ 0-3 สัปดาห์; 0.80 0.90 1.00 1.10 และ 1.20% ในช่วงอายุ 3-6 สัปดาห์ และ 0.69 0.79 0.89 0.99 และ 1.09% ในช่วงอายุ 6-9 และ 9-12 สัปดาห์ ผลการทดลองพบว่า ไก่โคราชมีการตอบสนองต่อระดับไลซีนในอาหาร โดยมีผลต่อการเพิ่มน้ำหนักตัว ประสิทธิภาพการใช้อาหาร กรดยูริกในพลาสมา การสะสมโปรตีนและกรดอะมิโนในทุกช่วงอายุ (ยกเว้นอาร์จีนีนในช่วงอายุ 0-3 และ 3-6 สัปดาห์) ($P<0.05$) เมื่อทำการประเมินความต้องการไลซีนที่ย่อยได้ของไก่โคราช ด้วยวิธีการวิเคราะห์แบบ Broken-line regression พบว่าความต้องการไลซีนของไก่โคราชที่เหมาะสมในช่วงอายุ 0-3 สัปดาห์ คือ 1.08 1.03 1.12 1.09 และ 1.10 %สำหรับการเพิ่มน้ำหนักตัว ประสิทธิภาพการใช้อาหาร กรดยูริก การสะสมโปรตีนและไลซีน ตามลำดับ ช่วงอายุ 3-6 สัปดาห์ คือ 0.97 1.00 0.95 0.96 และ 1.00 % สำหรับการเพิ่มน้ำหนักตัว ประสิทธิภาพการใช้อาหาร กรดยูริก การสะสมโปรตีนและไลซีน ตามลำดับ ช่วงอายุ 6-9 สัปดาห์ คือ 0.93 0.93 0.89 และ 0.90 สำหรับการเพิ่มน้ำหนักตัว ประสิทธิภาพการใช้อาหาร การสะสมโปรตีนและไลซีน ตามลำดับ และอายุ 9-12 สัปดาห์ คือ 0.82 0.87 0.88 0.84 และ 0.85% สำหรับการเพิ่มน้ำหนักตัว ประสิทธิภาพการใช้อาหาร กรดยูริก การสะสมโปรตีนและไลซีน ตามลำดับ โดยสรุปความต้องการไลซีนที่ย่อยได้สำหรับไก่โคราชเท่ากับในช่วงอายุ 0-3 3-6 6-9 และ 9-12 สัปดาห์ เท่ากับ 1.08 0.98 0.91 และ 0.85% ตามลำดับ โดยคิดจากค่าเฉลี่ยของทุกพารามิเตอร์ที่ทำการศึกษา

การทดลองที่ 2 เป็นการศึกษาความต้องการกรดอะมิโนซัลเฟอร์ของไก่โคราชช่วงอายุ 0-12 สัปดาห์ แบ่งออกเป็น 4 ระยะการทดลองตามช่วงอายุ 0-3 3-6 6-9 และ 9-12 สัปดาห์ ในแต่ละระยะการทดลองกำหนดให้มีกรดอะมิโนซัลเฟอร์ 5 ระดับ คือ 0.63 0.73 0.83 0.93 และ 1.03% ในช่วงอายุ 0-3 สัปดาห์; 0.58 0.66 0.74 0.82 และ 0.90% ในช่วงอายุ 3-6 สัปดาห์ และ 0.50 0.58 0.66 0.74 และ 0.82% ในช่วงอายุ 6-9 และ 9-12 สัปดาห์ ผลการทดลองพบว่า ไก่โคราชมีการตอบสนองต่อระดับกรดอะมิโนซัลเฟอร์ในอาหาร โดยมีผลต่อการเพิ่มน้ำหนักตัว ประสิทธิภาพการใช้อาหาร กรดยูริกในพลาสมา (ยกเว้นในช่วงอายุ 0-3 และ 3-6 สัปดาห์) การสะสมโปรตีนและกรดอะมิโนใน

ทุกช่วงอายุ (P<0.05) เมื่อทำการประเมินความต้องการกรดอะมิโนซัลเฟอร์ที่ย่อยได้ของไก่โคราช ด้วยวิธีการวิเคราะห์แบบ Broken-line regression พบว่าความต้องการไลซีนของไก่โคราชที่เหมาะสมในช่วงอายุ 0-3 สัปดาห์ คือ 0.83 0.81 0.85 0.85 และ 0.82% สำหรับการเพิ่มน้ำหนักตัว ประสิทธิภาพการใช้อาหาร กรดยูริก การสะสมโปรตีนและกรดอะมิโนซัลเฟอร์ ตามลำดับ ช่วงอายุ 3-6 สัปดาห์ คือ 0.74 0.76 0.75 และ 0.74% ตามลำดับ สำหรับการเพิ่มน้ำหนักตัว ประสิทธิภาพการใช้อาหาร การสะสมโปรตีนและกรดอะมิโนซัลเฟอร์ ตามลำดับ ช่วงอายุ 6-9 สัปดาห์ คือ 0.67 0.66 0.67 และ 0.68 สำหรับการเพิ่มน้ำหนักตัว ประสิทธิภาพการใช้อาหาร การสะสมโปรตีนและกรดอะมิโนซัลเฟอร์ ตามลำดับ และอายุ 9-12 สัปดาห์ คือ 0.65 0.66 0.66 และ 0.67% สำหรับการเพิ่มน้ำหนักตัว ประสิทธิภาพการใช้อาหาร การสะสมโปรตีนและกรดอะมิโนซัลเฟอร์ ตามลำดับ โดยสรุปความต้องการกรดอะมิโนซัลเฟอร์ที่ย่อยได้สำหรับไก่โคราชเท่ากับ 0.83 0.75 0.67 และ 0.66% ในช่วงอายุ 0-3 3-6 6-9 และ 9-12 สัปดาห์ ตามลำดับ โดยคิดจากค่าเฉลี่ยของทุกพารามิเตอร์ที่ทำการศึกษา

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TRAN HONG DINH : DIGESTIBLE LYSINE AND SULFUR AMINO ACID REQUIREMENTS OF KORAT CHICKENS. THESIS ADVISOR : ASSOC. PROF. SUTISA KHEMPAKA, Ph.D., 125 PP.

LYSINE/SULFUR AMINO ACID/REQUIREMENT/KORAT CHICKEN

This research aimed to estimate the lysine and sulfur amino acid requirements for Korat chickens from 0 to 12 wk of age, which were divided into 2 experiments.

Experiment I was conducted to estimate the lysine requirement for Korat chickens from 0 to 12 wk of age, which were divided into four periods (0-3 3-6 6-9 and 9-12 wk of age). In each period, five dietary lysine levels were composed of 0.87 0.97 1.07 1.17 and 1.27% in period 0-3 wk; 0.80 0.90 1.00 1.10 and 1.20% in period 3-6 wk; 0.69 0.79 0.89 0.99 and 1.09% in both periods 6-9 and 9-12 wk. The results showed the Korat chickens exhibited significant (P<0.05) responses to dietary lysine levels in body weight (BWXX) gain, feed conversion ratio (FCR), uric acid (UA) in plasma, protein deposition (PD), and amino acid deposition in all periods (except for Arg in periods 0-3 and 3-6 wk). The estimates of digestible lysine requirements using the broken-line regression analysis in period 0-3 wk were 1.08 1.03 1.12 1.09 and 1.10% for BW gain, FCR, UA, PD, and lysine deposition (LysD), respectively; in period 3-6 wk were 0.97 1.00 0.95 0.96 and 1.00% for BW gain, FCR, UA, PD, and LysD, respectively; in period 6-9 wk were 0.93 0.93 0.89 and 0.90 for BW gain, FCR, PD, and LysD, respectively; in period 9-12 wk were 0.82 0.87 0.88 0.84 and 0.85 for BW gain, FCR, UA, PD, and LysD, respectively. In conclusion, the estimated digestible lysine requirements for Korat chickens in periods 0-3 3-6 6-9 and 9-12 wk

were 1.08 0.98 0.91 and 0.85%, respectively based on the averages of measured criteria.

Experiment II was conducted to estimate the digestible sulfur amino acid (SAA) requirement for Korat chickens from 0 to 12 wk of age. The study consisted of four age periods (0-3 3-6 6-9 and 9-12 wk). In each period, five dietary sulfur amino acid levels were composed of 0.63 0.73 0.83 0.93 and 1.03% in period 0-3 wk; 0.58 0.66 0.74 0.82 and 0.90% in period 3-6 wk; 0.5, 0.58 0.66 0.74 and 0.82% in both periods 6-9 and 9-12 wk. The results indicated that the Korat chickens significantly (P<0.05) responded to dietary SAA levels in BW gain, FCR, UA in plasma (except in periods 3-6 and 6-9 wk), PD, and amino acid deposition in all periods. The estimates of digestible SAA requirements for Korat chickens were 0.83 0.81 0.85 0.85 and 0.82% for BW gain, FCR, UA, PD, and sulfur amino acid deposition (SAAD), respectively, in period 0-3 wk; 0.74 0.76 0.75 and 0.74% for BW gain, FCR, PD, and SAAD, respectively, in period 3-6 wk; 0.67 0.66 0.67 and 0.68% for BW gain, FCR, PD, and SAAD, respectively, in period 6-9 wk; and 0.65 0.66 0.66 and 0.67% for BW gain, FCR, PD, and SAAD, respectively, in period 9-12 wk. In brief, the digestible SAA requirements for Korat chickens were 0.83 0.75 0.67 and 0.66% in periods 0-3 3-6 6-9 and 9-12 wk, respectively, based upon averages of measured criteria.

School of Animal Technology and Innovation — Student's Signature Dinh

Academic Year 2020 — Advisor's Signature Sutisa Khempaka

Co-advisor's Signature W. Molee

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LIST OF ABBREVIATIONS

ANOVA	=	Analysis of variance
AOAC	=	Association of official analytical chemists
Arg	=	Arginine
BW	=	Body weight
BWG	=	Body weight gain
°C	=	Degree celsius
CP	=	Crude protein
CRD	=	Completely randomized design
Cys	=	Cystine
d	=	Day
DDGS	=	Distillers dried grains with solubles
FCR	=	Feed conversion ratio
FGB	=	Fast-growing broilers
FI	=	Feed intake
GLM	=	General linear model
h	=	Hour
His	=	Histidine
Ile	=	Isoleucine
Leu	=	Leucine
Lys	=	Lysine
LysD	=	Lysine deposition

LIST OF ABBREVIATIONS (Continued)

ME	=	Metabolizable energy
Met	=	Methionine
NE	=	Not estimated
NRC	=	National Research Council
PD	=	Protein deposition
Phe	=	Phenylalanine
SAA	=	Sulfur amino acids
SAAD	=	Sulfur amino acid deposition
SAS	=	Statistical analysis system
SEM	=	Standard error of the mean
SUT	=	Suranaree University of Technology
Thr	=	Threonine
Trp	=	Tryptophan
UA	=	Uric acid
Val	=	Valine
vs.	=	Versus
wk	=	Week
%	=	Percentage

CHAPTER I

INTRODUCTION

1.1 Description of problem

The steady increase in global demand for white meat has driven the poultry industry towards practices that increase production. The main reason for which white meat such as chicken meat is considered healthier in comparison with red meat (pork, beef, goat, etc.,) is that its relatively lower contents of fat, cholesterol, and iron (Jaturasitha et al., 2008). In addition, chicken is inexpensive commercially produced meat. However, this rapidly growing consumption of chicken meat is based on a few fast-growing broiler strains produced by commercial breeding companies in intensive fattening systems (Jaturasitha et al., 2008). A public debate on the use of fast-growing broilers (FGB) is currently ongoing in several countries (i.e., China, Italy, Japan, Botswana, Vietnam, and Thailand among others), there is a trend towards the production of poultry meat using slow-growing chickens (Wattanachant et al., 2004, 2005; Chen et al., 2008; Rikimaru and Takahashi, 2010; Kgwatalala et al., 2013; Lan Phuong et al., 2015; Zotte et al., 2019ab).

Based on an obvious practical demand, Korat chickens were established through a cross between the male Thai native chicken (Leung Hang Khao) and synthetic female broiler line (SUT line). Korat chickens can reach a marketable live weight of 1.3 kg at 63 days of age and 1.6 to 1.8 kg at 84 days (Hang et al., 2018ab; Maliwan et al., 2019). Interestingly, their meat has less fat but more protein and shear force (Yongsawatdigul et al., 2016), and higher antioxidant activity relative to commercial broilers (Sangsawad et al., 2016). Furthermore, Korat chickens can adapt well to hot temperatures when raised in an open-sided, naturally ventilated house. For these reasons, these birds are now widely distributed throughout Thailand although their price is much more 1.5-2.0 times compared to commercial broilers.

The issue is now how we can do to Korat chicken not only express the maximum genetic potential but also the minimum nitrogen excretion and low cost of feed. In fact, in addition to health and farm management factors, Korat chickens can only express their true genetic potential when the nutritional values match the gene. Metabolizable energy (ME) and crude protein (CP) requirements were determined from Maliwan (2016). However, dietary protein requirements are in fact requirements for the amino acids contained in the dietary protein because the protein is composed of the amino acid. There are certain benefits to formulating amino acid-based diets compared to CP, such as lower feed cost and nitrogen excretion. Therefore, it is extremely necessary to study amino acid requirements for Korat chickens; in particular, lysine and methionine requirements.

Generally, amino acid requirements of chickens can be influenced by dietary factors (protein level, energy level, and feed intake), environmental factors (disease, crowding, feeder space, and heat stress), and genetic factors (capacity for lean vs fat growth); however, the ideal ratio of indispensable amino acid to lysine should remain largely unaffected by these variables. There are three primary reasons why lysine is selected as an ideal amino acid profile although it is the second limiting amino acid. Firstly, unlike several other amino acids (methionine, cystine, and tryptophan), absorbed lysine is used only for protein accretion. Secondly, its analysis in feedstuffs, which differs from tryptophan and sulfur amino acids, is relatively simple and straightforward. Finally, there is extensive information on digestible lysine requirements in poultry (Baker and Han, 1994). For these reasons, lysine was selected as the reference amino acid for the "ideal protein" concept, which bases dietary amino acid concentrations on fixed ratios to lysine. In such diets, the change in lysine content will change the requirements for all other essential amino acids. As a result, it is important to obtain accurate estimates of lysine requirements.

Methionine plays an important role in poultry growth. It is the first limiting amino acid in common poultry diets. Dietary supplementation of methionine could improve growth performance and carcass quality of growing broiler chickens (Jensen et al., 1989; Hickling et al., 1990; Moran, 1994; Schutte and Pack, 1995; Esteve-Garcia and Llaurado, 1997; Wallis, 1999; Esteve-Garcia and Mack, 2000; Lemme et al., 2002; Vieira et al., 2004; Garcia and Batal, 2005; Lumpkins et al., 2007; Goulart et al., 2011; Conde-Aguilera et al., 2013). Methionine can be converted irreversibly to cysteine via transsulfuration; therefore, cystine can be considered a nonessential amino acid. Therefore, the requirements of these amino acids are generally considered together as requirements for methionine plus cystine (called sulfur amino acids, SAA). According to the ideal protein" concept, the SAA requirement for Korat chickens can be determined through SAA to Lys ratio. However, SAA still needs to be studied to estimate optimal levels for chickens for two main reasons. Firstly, the composition of the SAA can differ between the type of chicken-strain, as their feather percentage varies. Actually, Kalinowski et al. (2003) reported that Cys requirement for fast-feathering was higher than for slow-feathering. Secondly, the SAA requirement would be higher under hyper-thermoneutral conditions than under thermoneutral conditions (Silva et al., 2006; Bunchasak, 2009). Indeed, the SAA to Lys ratio was recommended in a range from 0.69 to 0.82 in previous reports (Baker and Han, 1994; NRC, 1994; Mack et al., 1999; Vieira et al., 2004; Goulart et al., 2011; Dozier and Mercier, 2013).

Regular determinations of amino acid requirements of broilers target on criteria of BW gain, FCR, and carcass components, in particular, breast meat yield to achieve the highest economic returns. In addition, poultry nutritionists also have concerned about the environmental issues caused by abdominal fat, which is considered to be the major source of waste at the slaughterhouse; therefore, some studies have estimated the lysine requirement on the basis of this criterion (Grisoni et al., 1991; Han and Baker, 1994; Attia, 2003). From a scientific viewpoint and environmental concern, the most logical measurement of the lysine/ SAA requirement should be based on lysine/ SAA accretion in the whole-body and UA in plasma as amino acid intake follows anabolic pathways to tissue proteins, hormones, neurotransmitters, and other bioactive molecules or catabolic pathways to the amino group and carbon skeleton. The amino group creates uric acid, whereas the carbon skeleton of some amino acids synthesizes fatty acid through Acetyl-CoA (Akers and Denbow, 2008). It means that a deficiency of lysine/ SAA in the diet will lead to a decrease in accretion of protein and lysine/ SAA in the whole-body, whereas the exceeded lysine/ SAA in the diet has no drawback effect on protein and lysine/ SAA accretion but it will cause an adverse impact on the environment besides increased diet cost. We therefore measured, next to growth performance, also UA in plasma and the accretion of protein/ amino acids in the whole-body of the Korat chickens.

1.2 Research objectives

1.2.1 To estimate the lysine requirement of Korat chickens in 4 periods from 1 to 84 days of age (I: 1-21 d, II: 22-42 d, III: 43-63 d, and IV: 64-84 days of age).

1.2.2 To estimate the SAA requirement of Korat chickens in 4 periods from 1 to 84 days of age (1-21, 22-42, 43-63, and 64-84 days of age).

1.3 Research hypothesis

The growth rate of Korat chickens is lower than those of commercial broilers. In addition, the proportion of modern genotype breast meat is also greater and has the highest lysine contents in carcass. Therefore, it was hypothesized that the lysine requirements of Korat chickens can be lower than those recommended for commercial broilers. In contrast, it was hypothesized that the SAA requirements for Korat chicken may be superior to the nutrient requirements of fast-growing broilers due to environmental conditions (i.e., tropical climate) and low feed consumption of Korat chickens.

1.4 Scope of the study

This study consists of two experiments. Particularly, experiment 1 estimates digestible lysine requirements and experiment 2 estimates digestible sulfur amino acid requirements for Korat chicken from 0 to 12 wk of age, which are divided into 4 experimental periods, namely 1-21, 22-42, 43-63, and 64-84 days of age. The parameters in both two experiments were measured as feed intake, body weight, body weight gain, feed conversion ratio, plasma uric acid, protein and amino acid accretion in the whole-body. Experiment 1 was conducted in cool temperature conditions (from

December 2017 to January 2018 for period I and from November 2018 to February 2019 for periods II, III, and IV), while experiment 2 was carried out in hot temperature conditions (from February 2019 to May 2019) in an open-sided, naturally ventilated house.

1.5 Expected benefits

1.5.1 Provide useful information for the establishment of an ideal amino acid profile for Korat chickens.

1.5.2 Provide a knowledge base on the lysine and SAA requirements of Korat chickens from 0 to 12 wk of age with four different periods (0-3 wk, 3-6 wk, 6-9 wk, and 9-12 wk).

1.5.3 This knowledge will provide a valuable tool to commercial Korat chickens and enable the use of an appropriate feed formulation to Korat chickens express the maximum genetic potential, but also the minimum nitrogen excretion and low feed cost.

1.5.4 Provide nutritional knowledge for feed formulation to other indigenous crossbred chicken strains not only in Thai but also in tropical countries.

1.5.5 These findings can become a useful reference point for researchers who are interested in a study on indigenous chicken and/or crossbred chicken strains in the future.

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CHAPTER II

LITERATURE REVIEW

2.1 The role of protein, amino acids in poultry production

In birds, protein plays the multiform role including structural, regulatory, and functional proteins. Structural proteins are found in feathers (approximately 20-30% of the body protein), bone, muscle, and skin. The regulatory role of protein is exemplified by enzymes, plasma proteins, and transport proteins. Proteins also play a functional role by supplying energy in the course of their degradation. There are clearly more similarities than differences in the protein metabolism of mammals and birds. In particular, the main difference is in the nature of the end products. The principal end product in mammals is urea whereas uric acid is a major compound in birds (Griminger and Scanes, 1986).

Protein is defined as long chains of amino acids linked by peptides bonds. Two amino acids create a dipeptide, three a tripeptide, and so forth, 10 or more linked amino acids are called polypeptides, and those with greater than about 50 amino acids are simply called proteins. Each of amino acids has unique properties due to the difference in the R groups. The sequence of amino acids produces polypeptide and protein chains with correspondingly varied and complex properties. Hence, 20 different amino acids with the variation of possible structures lead to very large functional properties (Akers and Denbow, 2008).

Dietary protein requirements are actually requirements for the amino acids contained in the dietary protein. Amino acids, dipeptides, and tripeptides resulting from digestion and absorption may serve a variety of metabolic functions and as precursors of many important nonprotein body constituents. Because body proteins are in a dynamic state, with synthesis and degradation occurring continuously, an adequate intake of dietary amino acids is required. If amino acids are inadequate, there is a reduction or cessation of growth and a withdrawal of protein from less vital body tissues to maintain the functions of more vital tissues. (NRC, 1994). Therefore, the role of amino acids is to synthetic tissue proteins, hormones, neurotransmitters, other bioactive molecules (D'Mello, 2003). In other words, amino acids are a major constituent of the biologically active compounds in the body. They exist in form of enzymes and hormones that play key roles in the physiology of any living organism (Wallis and Balnave, 1984).

There are 20 amino acids in body proteins. All amino acids are physiologically essential. According to nutritional view, these amino acids can be divided into two categories. One category is essential amino acids that poultry cannot synthesize at all or rapidly enough to meet metabolic requirements. The essential amino acids must be supplied by the diet. The other group is nonessential that can be synthesized from essential amino acids by poultry. Thus, the presence of sufficient amounts of nonessential amino acids in the diet reduces the necessity of synthesizing them from essential amino acids (NRC, 1994). Poultry requires the core include essential amino acids, namely methionine, lysine, threonine, arginine, valine, isoleucine, tryptophan, histidine, leucine, and phenylalanine (D’Mello, 2003). The difference from other animals, arginine is an essential amino acid for chickens due to the absence of a functional urea cycle in birds (Khajali and Wideman, 2010).

2.2 The importance of ideal protein and amino acid balance

Nowadays, the "ideal protein" concept used in feed formulation for poultry is based on balancing dietary amino acids to meet the requirement for maintenance and production. Hence, most research has focused on providing the ideal balance of amino acids. In this way, the nutritionist will get feed formulation for broilers with a balance of essential amino acids to rely on the choice of feed ingredients for providing adequate essential amino acids in diets (Burley et al., 2015) or supply of synthetic amino acids (Heger, 2003). Essential amino acids such as lysine, methionine, threonine, arginine, tryptophan, isoleucine, and valine are usually supplemented in broiler diets.

Another reason that needs to have to balance essential amino acids in chicken diets is an interrelation between essential amino acids, namely, lysine and arginine in the diets. Particularly, excess dietary lysine can increase the chick's arginine requirement. This is explained that increasing the dietary concentration of lysine leads to the stimulated activity of the arginine catabolizing enzyme (Nesheim, 1968). In short, it is very necessary to balance the essential amino acid in broiler diets to optimize profit through minimizing feed cost and maximizing performance.

2.3 Protein digestion

Chemical digestion of protein begins in the stomach by the action of pepsin. Pepsin that works optimally at a pH of 1.5-2.5 cleaves bonds involving tyrosine and phenylalanine. Pepsin digests around 10-15% of dietary protein before being inactivated in the lumen of the small intestine. In the small intestine, trypsin and chymotrypsin secreted by the pancreas break down proteins into peptides. Carboxypeptidase and aminopeptidase, both brush-border enzymes, cleave one amino acid at a time from the carboxyl and amino end of a polypeptide, respectively, whereas the other brush-border enzymes aminopeptidase and dipeptidase further cleave the protein (Akers and Denbow, 2008).

Table 2.1 Amino acid profiles expressed as percentages of lysine in chickens.

Amino acids	**NRC, 1984[1]**	**NRC, 1994[2]**	**Baker and Han, 1994[3]**	**Baker et al., 2002[4]**
Lys	100	100	100	100
Met	42	46	36	
Cys	36	36	36	
Met+Cys	78	82	72	
Thr	67	73	67	55.7
Arg	120	114	105	
Val	68	82	77	77.5
Ile	67	73	67	61.4
Try	19	18	16	16.6
His	29	32	37	
Phe	60	66	55	
Leu	113	109	111	
Tyr	52	56	50	
Pro	44	55	44	
Gly+Ser	124	114	65	

[1]Based on total amino acid

[2]Based on total amino acid

[3]Based on digestible amino acid

[4]Based on digestible amino acid

2.4 Metabolism of absorbed amino acids, dipeptides, and tripeptides

Previously, it was believed that only amino acids are absorbed. However, it is now well established that dipeptides and tripeptides are also actively absorbed in the small intestine of chickens. Metabolism of absorbed amino acids, dipeptides, and tripeptides is reviewed by Ganapathy et al. (1994) as below figure 1. In particular, oligopeptides that are hydrolyzed by gastric proteases and luminal peptidases continue to be hydrolyzed to small peptides and free amino acids by apical membrane-bound peptidases (1). Peptides are absorbed across the brush border membrane by PepT1 (2) whereas a small proportion of absorbed peptides are then absorbed intact across the basolateral membrane by a H^+-independent transport activity (3). The majority are hydrolyzed by intracellular peptidases (4). The resulting free amino acid, plus those absorbed across the apical membrane by a complement of Na^+-dependent and –independent amino acid transporters (5) are then transported across the basolateral membrane by a complement of Na^+-independent and amino acid exchanger transport protein (6). The extracellular-intercellular H^+ gradient that drives PepT1 activity, is re-established by the combined function of the apical Na^+/H^+ exchanger (7) and the basolateral Na^+/K^+ ATPase (8), which re-establishes the extracellular-intercellular Na^+ gradients diminished by both Na^+/H^+ exchanger and Na^+-coupled free amino acid transport. The contribution to total protein assimilation by free amino acid uptake from the lumen is represented by a composite transporter model (7), representing amino acid transport by Na^+-coupled, amino acid counter exchange, and/or facilitated transport proteins.

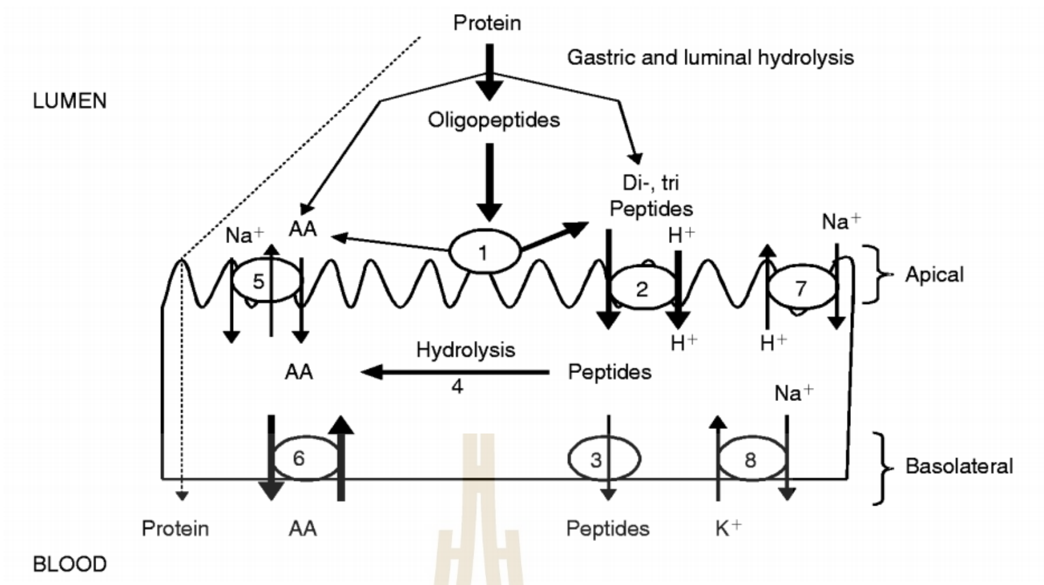

Figure 2.1 Metabolism of amino acid and peptide uptake.

2.5 Degradation of amino acids

Most of the amino acids over the requirements for protein synthesis and other biologically active substances are degraded in a cell- and tissue-specific manner. A major discrepancy between amino acids and other macronutrients (fat and carbohydrate) is amino acids containing nitrogen. In nature, N in substances exists in different oxidation forms: +5 (NO_3^-, nitrate), +3 (NO_2^-, nitrite, +2 (NO), 0 (N_2), −3 (NH_3, urea, and the –NH_2 group in amino acid). All of these states of N are produced by animals. Therefore, N actively participates in metabolic transformations in the body. The carbon skeletons of amino acids have a similar metabolic to glucose and fatty acids (Wu, 2013).

Due to differences inside chains, individual amino acids have their own unique catabolic pathways. Nevertheless, the catabolism of many amino acids shares a number of common steps to generate pyruvate, oxaloacetate, α-KG, fumarate, succinyl-CoA, and acetyl-CoA (Figure 2.2). Besides transamination, other reactions also play an important role in initiating amino acid degradation (Table 2.2). Complete degradation of many amino acids requires interorgan cooperation. Metabolites of amino acids composed of NH_3, CO_2, urea, uric acid, acetyl-CoA, short-chain fatty acids, formate, glucose, H_2S, ketone bodies, NO, polyamines, and other nitrogenous substances, with each having enormous biological importance (Wu, 2013).

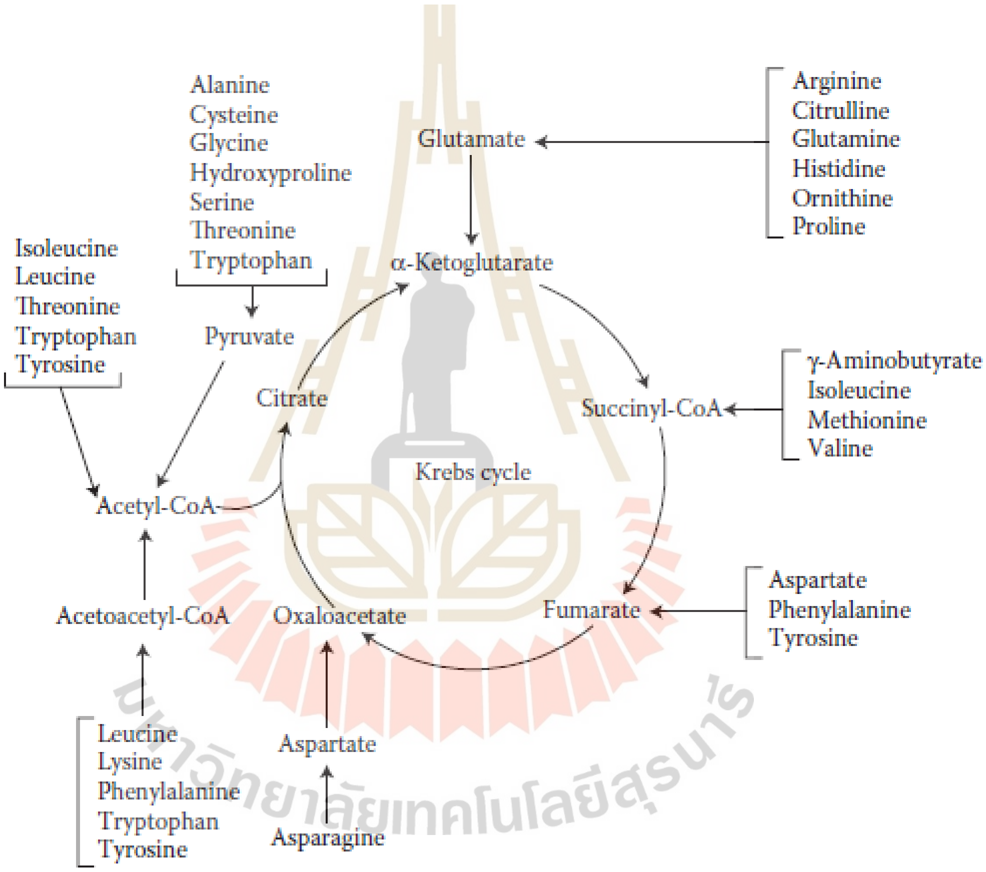

Figure 2.2 Different metabolic pathways for catabolism of amino acids converge to common intermediates that feed into the Krebs cycle in animals.

In birds, urates, ammonia, urea, and creatinine contribute to nitrogen excretion in the urine. Nevertheless, upon experiments with chickens, whether fed or fasted or provided low- or high-protein diets, urates constitute 55 to 84% of total nitrogen excretion. Thus, urates are the major waste product of nitrogen-containing metabolites excreted by the urinary system of birds. Birds excrete nitrogenous wastes as uric acid or the closely related compound guanine. The nitrogen group in uric acid are derived from the breakdown of glycine, aspartate, or glutamine. Birds lack the enzyme uricase and so can not break down uric acid (Akers and Denbow, 2008).

2.6 Digestible lysine and sulfur amino acid requirements of broilers

Body size and growth rate of poultry are determined by their genetics; therefore, amino acid requirements also vary among types, breeds, and strains of poultry. Genetic differences in amino acid requirements may occur because of differences in efficiency of digestion, nutrient absorption, and metabolism of absorbed nutrients (NRC, 1975). Although dietary requirements for amino acids usually are stated as percentages of the diet, the quantitative needs of poultry must be met by a balanced source to obtain maximum productivity. Thus factors that affect feed consumption also will affect quantitative intakes of amino acids and protein, and, consequently, will influence the dietary concentration of these nutrients needed to provide adequate nutrition (NRC, 1994).

Ambient temperature also affects feed intake of poultry (Hurwitz et al., 1980). Protein and amino acid requirements listed herein generally pertain to poultry kept at moderate temperatures (18° to 24°C). Ambient temperatures outside of this range cause an inverse response in feed consumption; that is, the lower the temperature, the greater the feed intake (NRC, 1981). Consequently, percentage requirements of protein and amino acids should be increased in warmer environments and decreased in cooler environments, in accordance with expected differences in feed intake. These adjustments

Table 2.2 Reactions initiating amino acid catabolism in animals.

Reactions	**Examples**
Amidotransferation	Glutamine + F6P $\rightarrow$ glucosamine-6-phosphate + glutamate
Cleavage	Glycine + NAD^+ + THF $\leftrightarrow$ MTHF + CO_2 + NH_3 + NADH + H^+
Condensation	Methionine + Mg-ATP $\rightarrow$ *S*-adenosylmethionine + Mg-PPi + Pi
Deaminated oxidation (FAD)	D-Amino acid + O_2 + H_2O $\rightarrow$ α-ketoacid + H_2O_2 + NH_3
	L-Amino acid + O_2 + H_2O $\rightarrow$ α-ketoacid + H_2O_2 + NH_3
Deamidation	Leucine + $1/2O_2$ $\rightarrow$ ketoacid $\rightarrow$ NH_3
Decarboxylation (PLP)	Ornithine $\rightarrow$ putrescine + CO_2
Dehydration	Serine $\rightarrow$ aminoacrylate + H_2O
Dehydrogenation	Threonine + NAD^+ $\rightarrow$ 2-amino-3-ketobutyrate + NADH + H^+
Dioxygenation	Cysteine + $O_2$$\rightarrow$ cysteinesulfinate
Hydrolysis	Arginine + H_2O $\rightarrow$ ornithine + urea
	Glutamine + H_2O $\rightarrow$ glutamate + NH_3
Hydroxylation	Arginine + O_2+ BH4 + NADPH + H^+ $\rightarrow$ NO + BH4 + citrulline + $NADP^+$
One-carbon unit transfer	Glycine + MTHF $\leftrightarrow$ serine + THF
Oxidation (FAD)	Proline + $1/2O_2$ $\rightarrow$ pyrroline-5-carboxylate + H_2O
Oxidative deamination	Glutamate + NAD^+ $\leftrightarrow$ α-ketoglutarate + NH_3 + NADH + H^+
Reduction	Lysine + α-ketoglutarate + NADPH + H^+ $\rightarrow$ saccharopine + $NADP^+$
Transamination (PLP)	Leucine + α-ketoglutarate $\leftrightarrow$ α-ketoisocaproate + glutamate

may aid in ensuring required daily intakes of amino acids. Some precautions, however, should be used in increasing the dietary protein concentration for poultry subjected to high ambient temperature. Waldroup et al. (1976) reported that performance of broiler chicks was improved by minimizing excess dietary amino acids.

2.6.1 Digestible lysine requirement of broilers

During the last three decades, a large number of scientific studies have been published on the lysine requirement of modern broiler (Table 2.3) and turkey strains (Lehmann et al., 1996; Boling and Firman, 1998). Nevertheless, knowledge of optimum dietary lysine concentrations for indigenous chickens is still scarce. Requirement published by NRC (1994) was not only derived from fast-growing broilers but also based on total lysine content, and these were partly based on experiments that were carried out more than 20 years ago.

Several studies use body weight gain as the single criterion to determine lysine requirements (NRC, 1994). However, there was evidence that shows digestible lysine requirement for breast meat yield was higher than those for feed conversion ratio and weight gain. In particular, digestible lysine requirement for breast meat yield is 1.16%, whereas feed conversion ratio is 1.07% and weight gain is 1.05% (Bernal et al., 2014). This may lead to an underestimation of the requirements of other parameters because weight gain requirements are usually lower than those for feed conversion ratio, breast yield, and abdominal fat, due to the same weight gain, lysine may reduce lipid deposition and increase protein accretion (Leclercq, 1998). Increasing lysine dietary concentration above the requirement for weight gain may result in higher breast yield. Therefore, lysine requirements are higher when breast yield is used instead of weight gain as the criterion to determine those requirements.

Feed conversion ratio is also frequently utilized to estimate amino acid requirements, and consequently, to calculate the ideal amino acid profile in dietary protein (Bernal et al., 2014). With the same viewpoint, Han and Baker (1993) also concluded that the lysine requirement for maximal feed efficiency was higher than that for maximal weight gain. Recent studies have used protein deposition (g) and body fat (g) were determined by comparative slaughter at the beginning and end of each feeding phase in order to investigate lysine requirement for broilers (Siqueira et al., 2013).

Up to now, there is two opposite point of views about the effect of lysine requirement on the sex of chickens. According to Han and Baker (1993), they concluded that male chicks required a higher level of dietary lysine than females for both maximal weight gain and feed efficiency. In contrast, Bernal et al., 2014 stated that there has no effect of lysine levels on the sex of chickens. They suggested digestible lysine requirements for maximum performance of Cobb 500 were determined as 1.22% for males and 1.24% for females in the starter phase, and 1.16% for both sexes in the grower phase. In addition, NRC (1994) recommended amino acid requirements for both males and females, not separate males and females.

2.6.2 Digestible lysine requirement of broilers

Methionine is the first limiting amino acid in chicken diets. Dietary methionine deficiency has been demonstrated to impair chicken growth (Corzo et al., 2006). This is explained that the growth process was regulated by skeletal muscle growth. It has been proven that skeletal muscle growth which is activated by amino acids, especially methionine (Dozier et al., 2008). Moreover, methionine deficiency also has been shown to result in lower breast muscle weight in broilers (Corzo et al., 2006), and there is a positive effect of increasing dietary methionine levels on chicken

Table 2.3 Digestible lysine requirments of broilers.

Strain of chicken	Age Period	Digestible Lys level (%)	Reference
Ross x Ross 708	1-7 d	1.35	Dozier and Payne (2012)
Hubbard x Cobb 500	1-7 d	1.26	Dozier and Payne (2012)
Ross x Ross	1-14 d	1.27	Dozier and Payne (2012)
Hubbard x Cobb 500	1-14 d	1.18	Dozier and Payne (2012)
Cobb 500	1-21 d	0.95 - 1.01	Garcia and Batal (2005)
Cobb 500	8-22 d	1.19	Siqueira et al. (2013)
New Hampshire x Columbian	8-21 d	1.21	Han and Baker (1991)
Hubbard x Hubbard	8-21 d	1.21	Han and Baker (1991)
Cobb 500	10-21 d	1.22-1.24	Bernal et al. (2014)
Ross x Ross TP16	14-28 d	1.00-1.10	Dozier et al. (2009)
Cobb 500	22-35 d	1.16	Bernal et al. (2014)
Ross × Ross	22-43 d	0.78- 0.85	Han and Baker (1994)
Ross × Ross TP16	28-42 d	0.99	Dozier et al. (2010)
Cobb × Cobb 700	28-42 d	0.97	Dozier et al. (2010)
Ross × Ross 308	42-56 d	0.85	Corzo et al. (2006)
Cobb × Cobb 500	1-12 d	1.03-1.04	Cemin et al. (2017)
Cobb × Cobb 500	12-28 d	0.85-0.88	Cemin et al. (2017)
Cobb × Cobb 500	28-42 d	0.84-0.86	Cemin et al. (2017)

breast muscle yield (Hickling et al., 1990). Increasing dietary methionine improved performance and breast muscle growth for broilers (Wen et al., 2014). Furthermore, under heat stress conditions, methionine supplementation could mitigate the effects of stress since methionine contributed to the increased expression levels of genes related to the antioxidant activity (Del Vesco et al., 2015). However, excess dietary methionine is detrimental to the immune response, growth performance, nutrient utilization, and organ characteristics of broiler chickens (Faluyi et al., 2015). For these reasons, it is very important to accurately calculate the level of methionine in feed formulation for broilers.

Digestible methionine requirement in the diet for male Ross x Ross 308 from 3-16 days of age was determined with 0.54% and digestible Met to digestible Lys ratio was 48 (Mehri et al., 2012). Estimating digestible sulfur amino acids for broiler chicks (Table 2.3) from 0 to 3 wk of age have ranged 0.67-0.88% in recent studies (Garcia and Batal, 2005; Goulart et al., 2011; Abdollahi et al., 2015), from 3 to 6 wk of age have ranged 0.55-0.75%, depending on the different strains and parameters.

Table 2.4 Digestible sulfur amino acid requirements of broilers.

Strain of chicken	Age Period	Levels (%)	SAA to Lys ratios	Reference
New Hampshirex Columbian	8-22 d	-	72	Baker and Han (1994)
Cobb 500, Ross 308	14-35 d	-	69	Vieira et al. (2004)
Ross × Ross 708	1-15 d	-	74	Dozier and Mercier (2013)
Hubbard × Cobb 500	1-15 d	-	77	Dozier and Mercier (2013
Ross × Hubbard	22-43 d	0.61	-	Baker et al. (1996)
Cobb 500	1-21 d	0.83-0.88	-	Garcia and Batal (2005)
Cobb 500	7-19 d	0.67	-	Lumpkins et al. (2007)
Cobb 500	21-42 d	0.56		Lumpkins et al. (2007)
Cobb	1-7 d	0.87	71	Goulart et al. (2011)
Cobb	8-21 d	0.76	70	Goulart et al. (2011)
Cobb	22-35 d	0.75	76	Goulart et al. (2011)
Cobb	36-42 d	0.66	72	Goulart et al. (2011)
Ross 308	1-10 d	0.69	-	Millecam et al. (2021)
Ross 308	11-23 d	0.66	-	Millecam et al. (2021)
Ross 308	24-35 d	0.62	-	Millecam et al. (2021)

2.7 Statistical models for estimating amino acid requirements

To estimate amino acid requirements for chickens, nutritionists often use a variety of methods such as multiple range tests, quadratic polynomials, broken line models with linear, broken line models with quadratic, the saturation kinetics model, a logistic model, and a compartmental model. However, each method exists advantages and disadvantages. In particular, The advantage of multiple range tests is easy to implement in software packages and seem relatively easy to understand, whereas the disadvantages of multiple range tests, the actual requirement can only be on or between two levels of the nutrient that were fed, and there is no way to tell precisely what it is. With quadratic polynomials (second order polynomials), easy to fit data (only three input levels are needed for quadratic responses, but three is not nearly enough points for the curve to be used to estimate the shape of the response or a requirement with any confidence). In contrast, second order polynomials are not able to have plateau levels of inputs between the requirement for maximum response level and levels that are toxic. The broken line models more closely represent theoretical ideas of the nature of nutritional responses than multiple range tests or polynomial models, but those models require more levels of nutrient input are needed to get good estimates. With other non-linear models as the saturation kinetics model, a logistic model, and a compartmental model, the advantage of these models is that more accurately depict biological responses than models that force responses to conform to straight lines. Nevertheless, they require more levels of nutrient input are needed to get good estimates. Besides, nonlinear models are more difficult to fit (Pest et al., 2009).

Vedenov and Pesti (2008) stated that no particular nonlinear model is necessarily best for all nutritional response data because the R^2 values for the various models did not differ very much. The choice of a model to evaluate data should depend on the objectives of the experiment (Baker, 1986). Many nutritionists estimated amino acid requirements for poultry are based on broken-line regression models because of 1) is relatively simple using standard nonlinear regression software, 2) provides a function that describes the response to nutrient dose across all dose levels, and 3) provides a break point estimate and standard error, interpreted as the nutrient requirement above which there is no significant change in the dependent variable (Robbins et al., 2006). A broken line model consists of two parts: a straight line with an increasing or decreasing slope and a horizontal line. Their point of intersection is the breakpoint. The linear broken-line model is often adequate for fitting growth data, especially if the levels tested cover a rather narrow range. For other types of biological data, the quadratic broken-line equation may be needed (Robbins, 1986).

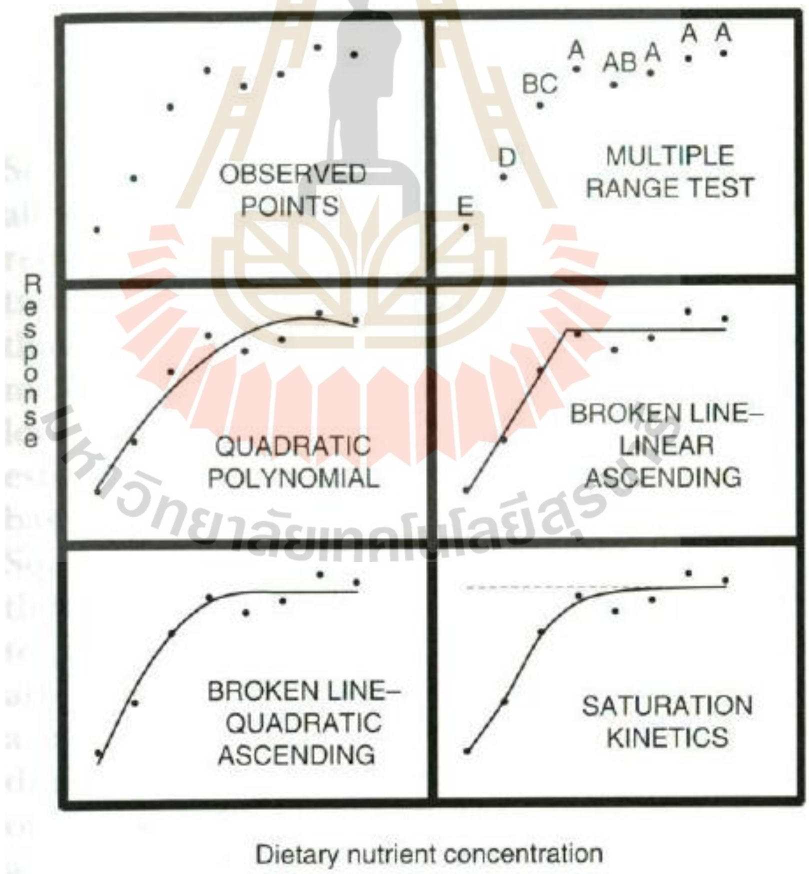

Figure 2.3 Representations of responses to dietary nutrient level.

Source: Pesti et al. (2009).

Indeed, several studies used the linear broken-line model to estimate lysine requirements (Han and Baker, 1991; Han and Baker, 1993; Han and Baker 1994; Mack et al., 1999; Labadan et al., 2001; Garcia and Batal, 2005; Garcia et al., 2006; Dozier and Mercier, 2013; Cemin et al., 2017) and methionine or sulfur amino acid requirements (Knowles and Southern, 1998; Garcia and Batal, 2005; Lumpkins et al., 2007; Millecam et al., 2021) whereas quadratic broken-line equation was used to estimate lysine and sulfur amino acid requirements in studies by Baker et al. (2002), Dozier et al. (2009); Dozier et al. (2010), Dozier and Payne (2012), Dozier and Mercier (2013), and Cemin et al. (2017).

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CHAPTER III

ESTIMATION OF LYSINE REQUIREMENTS FOR KORAT CHICKENS FROM 0 TO 12 WEEKS OF AGE

3.1 Abstract

This study was conducted to estimate the digestible lysine requirement of Korat chickens from 0 to 12 wk of age. The current study was divided into four age periods (I: 1-21 d; II: 22-42 d; III: 43-63 d; IV: 64-84 d). In each period, the chickens were randomly arranged to five dietary treatments with 6 replicates (18 birds per unit in the period I and 16 birds per unit in the other periods) in a completely randomized design. Experimental birds were fed 5 levels of digestible lysine: 0.87 0.97 1.07 1.17 and 1.27% in period I; 0.80 0.90 1.00 1.10 and 1.20% in period II; 0.69 0.79 0.89 0.99 and 1.09% in both period III and IV. The deposition of protein/amino acids was determined by the comparative slaughter technique. The results showed the Korat chickens exhibited significant (P<0.05) responses to dietary lysine levels in body weight (BW) gain, feed conversion ratio (FCR), uric acid (A) in plasma, protein deposition (D), and amino acid deposition (except for Arg in the period I and II) in all periods. The estimates of digestible lysine requirements using the broken-line regression analysis for BW gain, FCR, UA, PD, and lysine deposition (LysD) were 108 1.03 1.12 1.09 and 1.10% in period I; 0.97 1.00 0.95 0.96 and 1.00% in period II; 0.93 0.93 0.89 and 0.90 in period III, and 0.82 0.87 0.88 0.84 and 0.85 in period IV, respectively. In conclusion, the estimated digestible lysine requirements for Korat

chickens were 1.08 0.98 0.91 and 0.85% in periods I II III and IV, respectively, based on the averages of measured criteria.

Key words: lysine, crossbred broiler, growth performance, deposition

3.2 Introduction

Global poultry meat consumption is expected to reach to 140 million tonnes by 2028 (Shahbandeh, 2019). Besides fast-growing broilers, the consumption of slow-growing broilers has been increased greatly in recent years in several countries such as Italy, China, Japan, Botswana, and most Southeast Asian countries (Aini, 1990; Wattanachant et al., 2004; Chen et al., 2008; Zotte et al., 2019). This could be due to the slow-growing genotype that has unique features such as more palatable (high inosine 5′-monophosphate), high in shear force and collagen content, high n-3 fatty acids and low n-6/n-3 fatty acid ratio in meat lipids, high protein, low fat and cholesterol compared to the fast-growing genotype (Wattanachant et al., 2004; Jaturasitha et al. 2008; Rikimaru and Takahashi, 2010; Yongsawatdigul et al., 2016). Korat chicken, a new slow-growing broiler strain, has been crossed in Thailand between the male Thai native chicken (Leung Hang Khao) and the female modern genotype broiler chicken. The marketable live weight of KRC was about 1.3 kg at 63 days of age and from 1.6 to 1.8 kg at 84 days of age (Hang et al., 2018ab; Maliwan et al., 2019). Interestingly, its meat has less fat in addition to higher protein, shear force (Yongsawatdigul et al., 2016), and antioxidant activity than commercial broilers (Sangsawad et al., 2016).

In general, feeding should be targeted to enable birds to express the greatest potential of their genetic background. Energy and crude protein requirements of Korat chickens were determined by Maliwan et al. (2018, 2019). However, protein requirement is in fact requirements for the amino acids present in dietary protein. Formulating diets based on amino acid not only optimum growth performance but also minimum nitrogen excretion into the environment. Furthermore, the estimation of the precise lysine requirement has also gained importance, as such it is considered as a reference of other essential amino acids (Baker and Han, 1994; Baker et al., 2002). According to Baker and Han (1994), there are three primary reasons why lysine is selected as an ideal amino acid profile although it is the second limiting amino acid. Firstly, unlike several other amino acids (methionine, cystine, and tryptophan), absorbed lysine is used only for protein accretion. Secondly, its analysis in feedstuffs, different from tryptophan and sulfur amino acids, is relatively simple and straightforward. Finally, there is a considerable body of data on digestible lysine in poultry available. For these reasons, lysine was selected as a reference amino acid for the "ideal protein" concept, which bases dietary amino acid concentrations on fixed ratios to lysine.

Regular determinations of Lys requirements of broilers target on criteria of BW gain, FCR, and carcass components, especially the breast meat yield to achieve the highest economic returns. In addition, poultry nutritionists are also concerned about the environmental issue caused by abdominal fat, which is considered to be the main source of waste in the slaughterhouse; therefore, some studies have estimated the Lys requirement on the basis of this criterion (Grisoni et al., 1991; Han and Baker, 1994; Attia, 2003). Several studies indicated that the lysine requirement for BW gain is lower than those for breast meat, FCR, or abdominal fat (Grisoni et al., 1991; Han and Baker, 1991; Han and Baker, 1994; Leclercq, 1998; Garcia and Batal, 2005; Dozier III and Payne, 2012; Bernal et al., 2014). However, some studies reported that the lysine requirement for BW gain is higher than that for FCR (Labadan et al., 2001; Garcia et al., 2006; Cemin et al., 2017). From a scientific viewpoint and environmental concern, the most logical measurement of the lysine requirement should be based on lysine accretion in the whole-body and UA in plasma because amino acid intake follows anabolic routes to tissue proteins, hormones, neurotransmitters, and other bioactive molecules or catabolic pathways to the amino group and carbon skeleton. The amino group creates uric acid, whereas the carbon skeleton of some amino acids synthesize fatty acid through Acetyl-CoA (Akers and Denbow, 2008). It means that a deficiency of lysine in the diet will lead to a decrease in accretion of protein and lysine in the whole-body, whereas the exceeded lysine in the diet has no drawback effect on lysine accretion but it will cause an adverse impact on the environment besides increased diet cost.

3.3 Objective

For the above reasons, we carried out this study with the objectives to estimate optimum responses of Korat chickens to different dietary lysine levels based on growth performance, UA in plasma, PD, and LysD in the whole-body.

3.4 Materials and methods

3.4.1 Ethical considerations

All bird care and experimental procedures were approved by the Animal Ethics Committee of Suranaree University of Technology (Approval project number: SUT3-303-58-36-06) and based on the Ethics of Animal Experimentation of the National Research Council

3.4.2 Animals, housing and experimental design

The experiment was divided into 4 periods as 1-21, 22-42, 43-63, and 64-84 days of age in order to estimate the lysine requirements of Korat chickens. A total of 2,226 Korat chickens were used in the current study including 1,980 birds in dose-response, 66 birds in the comparative slaughter technique, and 180 birds in the determination of digestion. The birds were obtained from a hatchery belonging to SUT, Nakhon Ratchasima, Thailand. Since the gender of the Korat chickens cannot be ascertained one day after hatch, it was decided to use only un-sexed birds in the first period (1-21 days of age), whereas mixed-sex Korat chickens were used in the other periods (22-42, 43-63, and 64-84 days of age). The experimental birds were vaccinated against Marek's disease (FATRO S.p.A., Bologna, Italy) at the hatchery. On days 7 and 21, all birds were inoculated with Newcastle disease and infectious bronchitis vaccines (FATRO S.p.A., Bologna, Italy). On day 14, the birds were vaccinated against infectious bursal disease vaccine (FATRO S.p.A., Bologna, Italy). On day 28, they were inoculated with the flow pox vaccine (FATRO S.p.A., Bologna, Italy).

The experimental birds were raised in an open-sided, naturally ventilated barn, with a 23-h photoperiod using a fluorescent bulb as a light source, and the birds were housed on a concrete floor covered by rice husks disinfected with a disinfectant solution (glutaraldehyde). Brooding heat was provided by using an infrared heat lamp bulb 175 W above the birds. A brooding temperature of 35°C was provided for the first week and reduced by 3° every week. Each pen is equipped with a tray feeder and one drinker during the first 10 days of age. From day 11 onwards, nipple-type drinker lines and around-bottomed hanging feeders were used to supply feed. Both feed and water were available for ad libitum consumption throughout the experiment.

In the first period (1-21 days of age), a total of 654 hatchlings including 18 birds were killed upon arrival to determine the initial body composition and 540 were randomly allocated to 5 dietary treatments (Table 3.1) with 6 replicates of 18 birds per pen. The remaining birds were raised on the large floor pen to 12 days of age and fed on a diet containing 1.07% lysine (Table 3.1). On day 13, birds were randomly allocated to12 metabolic cages with 8 birds per unit. The birds still were fed a diet containing 1.07% lysine. On day 16, the birds were fed a nitrogen-free diet and a diet containing 0.87% lysine (Table 3.1) until day 20.

In the second period (22-42 days of age), a total of 532 hatchlings were initially fed a diet containing 2980 kcal ME/kg diet and 21.26% crude protein (as fed) from hatch to 21 d of age. On day 22, sixteen birds were used to determine the initial body composition and 480 mixed-sex birds (male and female are equal) were randomly allocated to 5 dietary treatments (Table 3.3) with 6 replicates of 16 birds per pen. The remaining birds were raised on the large floor pen until 33 days of age and fed on a diet containing 1.00% lysine (Table 3.3). On day 34, 36 mixed-sex birds were randomly allocated to metabolic cages with 6 replicates of 6 birds per unit. The birds still were fed a diet containing 1.00% lysine. On day 37, the birds were a diet containing 0.80% lysine (Table 3.3) until day 41.

In the third period (43-63 days of age), a total of 544 hatchlings were initially fed a diet containing 2980 kcal ME/kg t and 21.26% crude protein (as fed) from hatch to 21 d of age. Then, the birds were fed with a diet containing 3150 kcal ME/kg and 20.45% crude protein for up to 42 d of age. On days 43, sixteen birds were used to determine the initial body composition and 480 mixed-sex birds (male and female are equal) were randomly allocated to 5 dietary treatments (Table 3.5) with 6 replicates of 16 birds per pen. The remaining birds were raised on the large floor pen until 54 days of age and fed a diet that containing 0.89% lysine (Table 3.5). On day 55, 48 mixed-sex birds were randomly allocated to 12 metabolic cages with 4 birds per cage. The birds still were fed a diet containing 0.89% lysine. On day 58, the birds were fed a nitrogen-free diet and a diet containing 0.69% lysine (Table 3.5) until day 62.

In the final period (64-84 days of age), a total of 496 hatchlings were initially fed a diet containing 2980 kcal ME/kg diet and 21.26% crude protein (as fed) from hatch to 21 d of age. Subsequently, the birds were fed with a diet containing 3150 kcal ME/kg and 20.45% crude protein for up to 42 d of age. The birds were then fed with a diet containing 3200 kcal ME/kg and 18.00% crude protein up to 63 d of age. On days 64, sixteen birds were used to determine the initial body composition and 480 mixed-sex birds (male and female are equal) were randomly allocated to 5 dietary treatments (Table 3.5) with 6 replicates of 16 birds per pen.

3.4.3 Experimental diets

There were five dietary lysine levels in each trail period. Particularly, 0.87 0.97 1.07 1.17 and 1.27% lysine in the period I, 0.80 0.90 1.00 1.10 and 1.20% lysine in the period II, and 0.69 0.79 0.89 0.99 and 1.09% lysine in the period III and IV. The ingredients and chemical composition of the diets fed to Korat chickenswere presented in Table 3.1, Table 3.2, Table 3.3, Table 3.4, Table 3.5, and Table 3.6. The experimental diets were formulated to meet the requirements of metabolizable energy and crude protein recommended by Maliwan (2018, 2019). Other nutrient compositions in the diets were formulated to meet or exceed the nutrient requirements recommended by the NRC (1994), except for the lysine level. Both, L-glutamic acid and corn starch were used to replace variable levels of lysine to maintain isonitrogenous and isocaloric diets. The diets were fed in a mash form. Feedstuffs were analyzed for crude protein and total amino acid contents prior to feed formulation. The value of digestible amino acids in feedstuffs was determined using digestible coefficients (Ajinomoto Heartland LLC, 2009) and analyzed the total amino acid contents of the ingredients.

The nitrogen-free diet was formulated according to Adedokun et al. (2007), i.e., dextrose (64.00%), cornstarch (18.08%), cellulose (5.00%), soybean oil (5.00%), premix (0.50%), NaCl (0.20%), choline chloride (0.12%), calcium carbonate (1.50%), monocalcium phosphate (1.90%), $NaHCO_3$ (2.00%), KCl (1.20%), MgO (0.20%), titanium dioxide (0.30%).

3.4.4 Data and sample collection

The body weight of birds were recorded at the beginning and the end of the experiment. Feed refusals were collected at the end of the experiment. The deposition of proteins and amino acids in the whole-body was determined by the comparative slaughter technique at the beginning and the end of the experiment according to Wolynetz and Sibbald (1987). At the beginning of the experiment, 18 birds from period I, and 16 birds of each period II, III, and IV were killed with the use of chloroform (99.8%, RCI LABSCAN, Bangkok, Thailand) and the whole-body was subsequently stored at -20^{0}C until chemical analysis was performed. At the end of the experiment, 4 birds (2 males and 2 females) from period I, and 2 birds (1 male and 1 female)of each period II, III, and IV were selected on the basis of their body weight (birds with body weights closest to the pen mean) from each pen and housed for 24 h in metabolism cages so as to facilitate the fasting of the birds. During fasting, the animals were allowed unrestricted access to water. After the 24-h fasting period, the animals were killed as already described. Then, the whole-body was stored at -20^{0}C until the next step according to the procedure was described by Edwards et al. (1999). In brief, frozen whole-body of each unit was combined and chopped into small pieces, after which the combined pieces were ground three times. A 6-mm die was used for the first two grindings, and a 3-mm die was used for the third grinding. Following grinding, a subsample (approximately 300 g) of each unit was placed in freezer bags and frozen at -20^{0}C. Thereafter, the samples were freeze-dried (Gamma 2-16 LSC, Christ, UK), ground (blender, Philips, Netherlands) and stored at -20^{0}C until analysis.

At the end of each period (d 21, d 42, d 63, and d 84 of age), the feeders were removed for 2 h, then replaced the feeders to each pen for 20 minutes to ensure each bird has the opportunity to consume the feed. Next, those feeders were removed again. After 2 h of starvation, blood samples were collected from the bird's jugular or wing vein (four birds per pen including 2 males and 2 females) (Wilson and Miles, 1988; Donsbough et al., 2010). Blood samples were placed in 5-ml plastic polypropylene tubes containing lithium heparin and the samples were kept on the ice until centrifuged at 1,734×g at 0°C for 20 min. Plasma (0.25 ml) from each bird was collected and pooled by pen. The pooled plasma was subsequently sent to the hospital for uric acid analysis.

On days 20 (in period I), 41 (in period II), 62 (in period III), birds in metabolic cages were injected with Zoletil 100 (Virbac, Netherlands) andileal digesta was collected to determine ileal amino acid digestibility. The procedure was described by Kim et al. (2011). Briefly, the contents of the ileum were considered to be the portion of the small intestine from the Meckel's diverticulum to approximately the 3-cm proximal to the ileo-cecal junction. The ileal digesta was freeze-dried, grind by using a mortar and pestle to pass through a 0.5 mm sieve and samples was stored at -20°C for subsequent analyses.

3.4.5 Chemical analysis

The dry matter and crude fiber contents of the experimental diets were determined according to the AOAC (1990) procedures ID 930.15 and ID 962.09, respectively. The nitrogen and ether extract contents were analyzed according to the AOAC 2006 procedures ID 990.03 and ID 2003.05, respectively. The ash content was determined according to Thiex and Novotny (2012). Titanium dioxide in the diets and ileal digesta were analyzed according to Short et al. (1996).

Amino acids in feedstuffs, diets, ileal digesta, and the whole-body of birds, were analyzed according to AOAC (2000, ID 994.12). Briefly, performic acid was used to oxidize cystine and methionine to cysteic acid and methionine sulfone, respectively. Next, the samples were hydrolyzed by means of HCl (6 M) phenol solution (Multiwave 3000, Anton Paar GmbH, London, UK) under nitrogen gas at a temperature of 150^0C for 30 min. Norleucine ((Sigma-Aldrich, St Louis, MO) was used as an internal standard. oxidised hydrolysated standard includes 23 amino acids such as L-cysteic acid, taurine, D,L-methionine sulfoxide, L-methionine sulfone, L-aspartic acid, L-threonine, L-serine, L-glutamic acid, L-proline, glycine, L-cystine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-histidine, L-ornithine, L-lysine, ammonia, L-arginine (Biochrom 30+, Cambridge, UK) were used. Separation of amino acids was achieved with the use of an amino acid analyzer (Biochrom 30+, Cambridge, UK) using appropriate sodium buffers and ninhydrin reagent (Biochrom 30+, Cambridge, UK).

3.4.6 Statistical Analysis

The data were subjected to ANOVA using the general linear model procedure in SAS software (SAS Institute, 1996). When the effect of dietary treatments showed statistical significance, Tukey's test was used to identify the specific effect of each Lys level. Statistical significance was set at the level of $P \leq 0.05$. The broken-line regression analysis was used to estimate the optimum dietary Lys levels using the NLIN procedure of SAS software (SAS Institute, 1996) based on Robbins et al. (2006). The broken-line regression analysis was fitted as $Y = L + U \times (R - x)$, where Y is the dependent variable; x is the dietary Lys level as independent variable; R is the optimum response of dietary Lys; L is the response at x = r; and U = the steepness of the curve.

3.4.7 Experimental location

The experiment was conducted at Suranaree University of Technology's poultry farm, the Center for Scientific and Technological Equipment Building 10, Suranaree University of Technology.

3.4.8 Experimental period

The experiment was done from December 2017 to January 2018 for period I (1-21 days of age) and from November 2018 to February 2019 for periods II, III, and IV.

Table 3.1 The ingredient of the experimental diets fed to Korat chickens in period I (1-21 days of age).

	Digestible lysine level				
Item	**0.87%**	**0.97%**	**1.07%**	**1.17%**	**1.27%**
Ingredient, %					
Corn	56.81	56.81	56.81	56.81	56.81
Soybean meal	25.01	25.01	25.01	25.01	25.01
Corn DDGS	8.00	8.00	8.00	8.00	8.00
Rice bran oil	1.24	1.24	1.24	1.24	1.24
Calcium carbonate	1.79	1.79	1.79	1.79	1.79
Monocalcium phosphate	1.54	1.54	1.54	1.54	1.54
Sodium chloride	0.48	0.48	0.48	0.48	0.48
Premix[1]	0.50	0.50	0.50	0.50	0.50
Glutamic acid, purity 99%	1.41	1.21	1.01	0.81	0.61
Cornstarch	1.61	1.68	1.76	1.83	1.91
DL-Met, purity 99%	0.48	0.48	0.48	0.48	0.48
L-Lys HCl, purity 78%	0.00	0.13	0.25	0.38	0.50
L-Thr, purity 98.5%	0.41	0.41	0.41	0.41	0.41
L-Arg, purity 99%	0.33	0.33	0.33	0.33	0.33
L-Ile, purity 99%	0.18	0.18	0.18	0.18	0.18
L-Val, purity 99%	0.16	0.16	0.16	0.16	0.16
L-Trp, purity 98.5%	0.05	0.05	0.05	0.05	0.05
Total, %	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

[1]Premix (0.5%) provided the following (per kg of diet): vitamin A, 15,000 IU; vitamin D_3, 3000 IU; vitamin E, 25 IU; vitamin K_3, 5 mg; vitamin B_1, 2 mg; vitamin B_2, 7 mg; vitaminB_6, 4 mg; vitaminB_{12}, 25 mg; pantothenic acid, 11.04 mg; nicotinic acid, 35 mg; folic acid, 1 mg; biotin, 15 μg; choline chloride, 250 mg; Cu, 1.6 mg; Mn, 60 mg; Zn, 45 mg; Fe, 80 mg; I, 0.4 mg; Se, 0.15 mg.

Table 3.2 Chemical composition of the diets fed to Korat chickens in period I (1-21 days of age). Unless otherwise indicated, values are expressed as % as fed.

	Digestible lysine level				
Item	**0.87%**	**0.97%**	**1.07%**	**1.17%**	**1.27%**
Calculated composition					
ME, kcal/kg	2980	2980	2982	2980	2984
Crude protein	21.26	21.26	21.26	21.26	21.26
Digestible Lys[1]	0.87	0.97	1.07	1.17	1.27
Digestible Met	0.76	0.76	0.76	0.76	0.76
Digestible Met + Cys	1.04	1.04	1.04	1.04	1.04
Digestible Thr	0.93	0.93	0.93	0.93	0.93
Digestible Arg	1.45	1.45	1.45	1.45	1.45
Digestible Ile	0.93	0.93	0.93	0.93	0.93
Digestible Val	1.04	1.04	1.04	1.04	1.04
Digestible Leu	1.62	1.62	1.62	1.62	1.62
Digestible His	0.58	0.58	0.58	0.58	0.58
Digestible Phe	0.83	0.83	0.83	0.83	0.83
Digestible Trp	0.23	0.23	0.23	0.23	0.23
Ca	1.02	1.02	1.02	1.02	1.02
Available P	0.46	0.46	0.46	0.46	0.46
Analyzed composition					
Dry matter	89.85	89.88	89.83	89.76	89.65
Crude protein	20.74	20.65	20.85	21.20	21.19
Crude fiber	2.89	2.95	2.94	2.90	2.93
Ether extract	4.18	4.27	4.20	4.23	4.13
Crude ash	5.28	5.29	5.25	5.24	5.25

[1]Digestible amino acid values of the diets were calculated using the digestibility coefficients reported by Ajinomoto Heartland LLC (2009) for the individual feedstuffs (i.e., corn, soybean meal, and corn DDGS) while digestibility coefficients of synthetic amino acids were assumed as 100%.

Table 3.3 The ingredient of the experimental diets fed to Korat chickens in period II (22-42 days of age).

	Digestible lysine level				
Item	**0.80%**	**0.90%**	**1.00%**	**1.10%**	**1.20%**
Ingredient, %					
Corn	60.98	60.98	60.98	60.98	60.98
Soybean meal	22.90	22.90	22.90	22.90	22.90
Corn gluten meal	3.50	3.50	3.50	3.50	3.50
Rice bran oil	2.17	2.17	2.17	2.17	2.17
Calcium carbonate	1.68	1.68	1.68	1.68	1.68
Monocalcium phosphate	1.18	1.18	1.18	1.18	1.18
Sodium chloride	0.43	0.43	0.43	0.43	0.43
Premix[1]	0.50	0.50	0.50	0.50	0.50
Glutamic acid, purity 99%	1.79	1.59	1.39	1.18	0.98
Cornstarch	3.55	3.62	3.70	3.78	3.85
DL-Met, purity 99%	0.30	0.30	0.30	0.30	0.30
L-Lys HCl, purity 78%	0.00	0.13	0.25	0.38	0.51
L-Thr, purity 98.5%	0.39	0.39	0.39	0.39	0.39
L-Arg, purity 99%	0.28	0.28	0.28	0.28	0.28
L-Ile, purity 99%	0.17	0.17	0.17	0.17	0.17
L-Val, purity 99%	0.13	0.13	0.13	0.13	0.13
L-Trp, purity 98.5%	0.05	0.05	0.05	0.05	0.05
Total, %	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

[1]Premix (0.5%) provided the following (per kg of diet): vitamin A, 15,000 IU; vitamin D_3, 3,000 IU; vitamin E, 25 IU; vitamin K_3, 5 mg; vitamin B_1, 2 mg; vitamin B_2, 7 mg; vitamin B_6, 4 mg; vitamin B_{12}, 25 mg; pantothenic acid, 11.04 mg; nicotinic acid, 35 mg; folic acid, 1 mg; biotin, 15 μg; choline chloride, 250 mg; Cu, 1.6 mg; Mn, 60 mg; Zn, 45 mg; Fe, 80 mg; I, 0.4 mg; Se, 0.15 mg.

Table 3.4 Chemical composition of the diets fed to Korat chickens in period II (22-42 days of age). Unless otherwise indicated, values are expressed as % as fed.

	Digestible lysine level				
Item	**0.80%**	**0.90%**	**1.00%**	**1.10%**	**1.20%**
Caculated composition					
ME, kcal/kg	3150	3152	3153	3154	3155
Crude protein	20.45	20.45	20.45	20.45	20.45
Digestible Lys[1]	0.80	0.90	1.00	1.10	1.20
Digestible Met	0.58	0.58	0.58	0.58	0.58
Digestible Met + Cys	0.86	0.86	0.86	0.86	0.86
Digestible Thr	0.89	0.89	0.89	0.89	0.89
Digestible Arg	1.32	1.32	1.32	1.32	1.32
Digestible Ile	0.88	0.88	0.88	0.88	0.88
Digestible Val	0.98	0.98	0.98	0.98	0.98
Digestible Leu	1.68	1.68	1.68	1.68	1.68
Digestible His	0.55	0.55	0.55	0.55	0.55
Digestible Phe	0.84	0.84	0.84	0.84	0.84
Digestible Trp	0.22	0.22	0.22	0.22	0.22
Ca	0.90	0.90	0.90	0.90	0.90
Available P	0.35	0.35	0.35	0.35	0.35
Analyzed composition					
Dry matter	91.11	91.24	91.32	91.44	91.24
Crude protein	20.39	20.27	20.34	20.30	20.40
Crude fiber	2.09	2.17	2.12	2.01	2.06
Ether extract	4.86	4.89	4.86	4.86	4.80
Crude ash	5.25	5.22	5.27	5.29	5.28

[1]Digestible amino acid values of the diets were calculated using the digestibility coefficients reported by Ajinomoto Heartland LLC (2009) for the individual feedstuffs (i.e., corn, soybean meal, and corn gluten meal) while digestibility coefficients of synthetic amino acids were assumed as 100%.

Table 3.5 The ingredient of the diets fed to Korat chickens in period III (43-63 days of age) and period IV (64-84 days of age).

Item	Digestible lysine level				
	0.69%	**0.79%**	**0.89%**	**0.99%**	**1.09%**
Ingredient, %					
Corn	62.07	62.07	62.07	62.07	62.07
Soybean meal	20.40	20.40	20.40	20.40	20.40
Corn gluten meal	3.20	3.20	3.20	3.20	3.20
Rice bran oil	2.51	2.51	2.51	2.51	2.51
Calcium carbonate	1.57	1.57	1.57	1.57	1.57
Monocalcium phosphate	0.99	0.99	0.99	0.99	0.99
Sodium chloride	0.44	0.44	0.44	0.44	0.44
Premix[1]	0.50	0.50	0.50	0.50	0.50
Glutamic acid, purity 99%	0.80	0.60	0.40	0.20	0.00
Cornstarch	6.07	6.14	6.22	6.29	6.37
DL-Met, purity 99%	0.26	0.26	0.26	0.26	0.26
L-Lys HCl, purity 78%	0.00	0.13	0.25	0.38	0.50
L-Thr, purity 98.5%	0.43	0.43	0.43	0.43	0.43
L-Arg, purity 99%	0.37	0.37	0.37	0.37	0.37
L-Ile, purity 99%	0.18	0.18	0.18	0.18	0.18
L-Val, purity 99%	0.15	0.15	0.15	0.15	0.15
L-Trp, purity 98.5%	0.06	0.06	0.06	0.06	0.06
Total, %	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

[1]Premix (0.5%) provided the following (per kg of diet): vitamin A, 15,000 IU; vitamin D_3, 3,000 IU; vitamin E, 25 IU; vitamin K_3, 5 mg; vitamin B_1, 2 mg; vitamin B_2, 7 mg; vitamin B_6, 4 mg; vitamin B_{12}, 25 mg; pantothenic acid, 11.04 mg; nicotinic acid, 35 mg; folic acid, 1 mg; biotin, 15 µg; choline chloride, 250 mg; Cu, 1.6 mg; Mn, 60 mg; Zn, 45 mg; Fe, 80 mg; I, 0.4 mg; Se, 0.15 mg.

Table 3.6 Chemical composition of the diets fed to Korat chickens in period III (43-63 days of age) and period IV (64-84 days of age). Unless otherwise indicated, values are expressed as % as fed.

	Digestible lysine level				
Item	**0.69%**	**0.79%**	**0.89%**	**0.99%**	**1.09%**
Calculated composition					
ME, kcal/kg	3200	3201	3202	3203	3204
Crude protein	18.00	18.00	18.00	18.00	18.00
Digestible Lys[1]	0.69	0.79	0.89	0.99	1.09
Digestible Met	0.52	0.52	0.52	0.52	0.52
Digestible Met + Cys	0.77	0.77	0.77	0.77	0.77
Digestible Thr	0.87	0.87	0.87	0.87	0.87
Digestible Arg	1.28	1.28	1.28	1.28	1.28
Digestible Ile	0.80	0.80	0.80	0.80	0.80
Digestible Val	0.90	0.90	0.90	0.90	0.90
Digestible Leu	1.51	1.51	1.51	1.51	1.51
Digestible His	0.48	0.48	0.48	0.48	0.48
Digestible Phe	0.74	0.74	0.74	0.74	0.74
Digestible Trp	0.20	0.20	0.20	0.20	0.20
Ca	0.82	0.82	0.82	0.82	0.82
Available P	0.31	0.31	0.31	0.31	0.31
Analyzed composition					
Dry matter	91.38	91.52	91.52	91.51	91.53
Crude protein	18.19	18.00	18.20	18.17	17.97
Crude fiber	1.96	2.02	1.98	2.05	1.93
Ether extract	5.19	5.09	5.12	5.11	5.07
Crude ash	4.96	5.02	4.93	5.06	4.93

[1]Digestible amino acid values of the diets were calculated using the digestibility coefficients reported by Ajinomoto Heartland LLC (2009) for the individual feedstuffs (i.e., corn, soybean meal, and corn gluten meal) while digestibility coefficients of synthetic amino acids were assumed as 100%.

3.5 Results and discussion

3.5.1 Period I (1-21 days of age)

Standardized digestible amino acid values in the basal dietare shown in Table 3.7. The result indicated that the values of Lys, Thr, Arg, Ile, Val, Leu, His, and Phe were close to the calculated true digestible values from analyzed total amino acid with digestible coefficients reported by Ajinomoto Heartland LLC (2009) (Table 3.2), whereas the value of Met was higher but Cys was lower than those calculated values in Table 3.2. However, the value of Met+Cys was similar to the calculated value.

Table 3.7 Standardized digestible amino acid values of the diets provided to Korat chickens in period I, II, III, and IV. Values are expressed as % as fed.

Item	Period I (1-21 days of age)	Period II (22-42 days of age)	Period III and IV (43-63 and 64-84 days of age)
Lys	0.86	0.80	0.70
Met	0.80	0.62	0.52
Cys	0.25	0.26	0.23
Thr	0.93	0.94	0.87
Arg	1.41	1.30	1.22
Ile	0.89	0.72	0.74
Val	1.02	0.78	0.76
Leu	1.55	1.63	1.38
His	0.58	0.58	0.51
Phe	0.84	0.86	0.72

The mortality in this period was found to be 0.74% and was unrelated to a specific dietary treatment. The Korat chickens significantly responded to increasing dietary lysine concentrations in final BW (P=0.013), BW gain (P=0.014), FCR (P<0.001), and UA in plasma (P<0.001), whereas no significant differences were observed in FI (P=0.794) between experimental treatments (Table 3.8). Protein deposition in the whole-body also significantly increased (P=0.001) as the dietary lysine level increased (Table 3.9). Likewise, increasing dietary lysine levels significantly improved ($P \leq 0.04$) the deposition of essential amino acids (Lys, Met, Thr, Ile, Val, Leu, His, and Phe), except Arg (P=0.516) (Table 3.9).

Table 3.8 Growth performance of Korat chickens from 1 to 21 days of age fed different digestible lysine levels. Unless otherwise indicated, values are expressed as g/bird.

Item	Digestible lysine level					SEM	*P*-value
	0.87%	0.97%	1.07%	1.17%	1.27%		
Body weight							
Initial	45.67	45.74	45.83	45.83	45.85	0.049	0.760
Final	271.3^{b}	301.5^{ab}	313.5^{a}	315.6^{a}	320.0^{a}	5.262	0.013
Gain	225.7^{b}	255.7^{ab}	267.7^{a}	269.7^{a}	274.2^{a}	5.263	0.014
Feed intake	442.1	437.1	430.3	428.7	418.4	5.979	0.794
FCR, g/g	1.96^{a}	1.72^{b}	1.62^{b}	1.59^{b}	1.53^{b}	0.035	<0.001
Uric acid, mg%	7.80^{a}	6.25^{ab}	4.94^{bc}	4.95^{bc}	3.52^{c}	0.322	<0.001

$^{a, b, c}$Means within each row with different superscripts are significantly different ($P \leq 0.05$).

BWG = body weight gain; FCR = feed converion ratio; SEM = standard error of the mean.

The alteration of dietary lysine content did not affect the FI of Korat chickens. This is probably because all diets were formulated to contain a similar ME concentration (from 2980 to 2984 kcal ME/kg). In general, the energy content in diets is the most important factor in regulatingthe FI of birds to meet their metabolic energy requirement (MacLeod, 1997).This statement is corroborated by Maliwan et al. (2018), who indicated that FI of Korat chickens reduces significantly within a range of 2750 to 3200 kcal ME/kg to keep their energy intake constant. Besides the energy factor, FI is also affected by other factors such as temperature, relative humidity, stocking density, water supply, and dietary fiber content (Kondra et al., 1974; Ross et al., 1981; Feddes et a., 2002; Lin et al., 2006; Ferket and Gernat, 2006). These factors were controlled to be similar in all treatments in the current study. The constant of FI in this study is in agreement with some previous studies (Grisoni et al., 1991; Siqueira et al., 2013), who found that FI was independent from the various dietary lysine contents, whereas this result is in contrast with findings of other studies (Tesseraud et al., 1992; Han and Baker, 1994; Fatufe et al., 2004), which revealed that chicks reduced their FI when offered a diet deficient in lysine, even though ME content of all experimental diets was also similar. Overall, the different effects of dietary lysine on FI depend on strains of chickens (Dozier III et al., 2010; Dozier III and Payne, 2012) or differences in growth rate (Dozier III et al., 2009; Bernal et al., 2014).

We observed a significant reduction in BW gain in birds fed a diet containing 0.87% digestible lysine compared to 1.07, 1.17, and 1.27%. Itindicated this lysine level contains an insufficient amount to ensure protein accretion to further achieve optimum BW gain of Korat chickens. The decline in growth rate associated with a diet diffienct in lysine could be explained by either a lower protein synthesis rate or higher degradation rate, or simultaneous alteration of both components of protein turnover in the whole-body (Roeder and Broderick, 1981; Muramatsu et al., 1986; Tesseraud et al., 1992, 1996; Urdaneta-Rincon and Leeson, 2004). A significant improvement in FCR relative to increasing dietary lysine concentrations is due to a significant increase in BW gain but not in FI. Dietary lysine deficiency leads to a significant reduction in BW gain and a negative impact on FCR is consistent with previous studies (Han and Baker, 1991; Fatufe et al., 2004; Garcia and Batal, 2005; Dozier et al., 2009, 2010, 2012; Bernal et al., 2014; Cemin et al., 2017).

Besides the growth performance, UA in plasma is also a viable criterion to determine amino acid requirements of broilers or the efficiency of amino acid utilization (Miles and Featherston, 1974; Donsbough et al., 2010) because UA, and not urea, is produced as the main end product of nitrogen metabolism in birds (Akers and Denbow, 2008). The data in this period clearly show that the UA reduced significantly with increasing dietary lysine levels. Uric acid reached the highest level when birds fed the basal diet (0.87% digestible lysine) and significantly higher compared to UA of birds fed the diets containing 1.07 1.17 and 1.27% digestible lysine. This can be explained that all experimental diets were formulated with the constant of essential amino acid content except for lysine. Therefore, the utilization efficiency of these amino acids is limited due to a dietary lysine deficiency. As a result,birds fedon diets containing inadequate lysine levels (0.87 and 0.97%) lead to higher UA than birds fed on diets containing adequate or exceed lysine requirements (1.07 1.17 and 1.27%). Obviously, UA in plasma decreased and BW gain increased as dietary lysine was increased to the required level, only slight changes occurring as the dietary lysine levels increased beyond the requirement. This finding is consistent with the reports by Miles and Featherston (1974) and Donsbough et al. (2010).

The increase in dietary lysine from moderate to high levels (1.07, 1.17, and 1.27%) showed no significant differences in PD and LysD among treatments, whereas there were significant improvements compared to insufficient level (0.87%). In this respect, these results are in line with findings on BW gain and UA excretion. These findings were also in line with the reports of Fatufe et al. (2004) and Siqueira et al. (2013), indicating that protein/ amino acids accretion of birds (Ross and Lohmann White in the study by Fatufe et al. (2004) and Cobb 500 chickens in the study by Siqueira et al. (2013) fed with increasing lysine contents (from 0.38 to 1.68% in the study by Fatufe et al. (2004) and from 0.84 to 1.21% in the study by Siqueira et al. (2013). This maybe due to a greater increase in the protein synthesis rate than in the degradation rate in dietary lysine from moderate to high levels (1.07 1.17 and 1.27%) compared to insufficient level (0.87%). However, an excess supply of lysine over requirement resulted in no further significant improvement in protein synthesis or degradation (Salter et al., 1990). For obvious reasons, the data in the current study indicated the diet of 1.07% lysine is adequate for maximum PD/LysD in the whole-body of Korat chickens.

Using the broken-line regression analysis, the digestible lysine requirement of Korat chickens was estimated at 1.08 1.03 1.12 1.09 and 1.10% for BW gain, FCR, UA, PD, and LysD, respectively (Table 3.16). The digestible lysine requirements of Korat chickens in the current study were very close to the digestible lysine requirements of Cobb 500 at 21 d of age (1.01 and 1.10% for BW gain and gain:feed ratio, respectively) (Garcia and Batal, 2005). In addition, Cemin et al. (2017) also estimated the optimum lysine levels of Cobb × Cobb 500 male broilers for BW gain and FCR as 1.14% and 1.12% (from 1 to 12 days of age) and 0.96 and 1.03% (from 12 to 28 days of age), respectively. Han and Baker (1991) also reported that digestible lysine requirements of Hubbard x Hubbard from 8 to 21 days of age for BW gain and FCR were 1.01 and 1.21%, respectively. However, the optimum lysine levels for Korat chickens in the present research were lower than those for fast-growing broilers in some previous reports. For example, Dozier III and Payne (2012) stated that digestible lysine requirements of female Hubbard x Cobb 500 from 1 to 15 days of age based on BW gain and FCR were 1.18% and 1.26%, respectively. Siqueira et al. (2013) found the digestible lysine requirements of Cobb 500 from 8 to 22 days of age were 1.19% based upon FCR. Moreover, Bernal et al. (2014) reported that digestible lysine requirements of Cobb 500 from 10-21 days of age were 1.19% and 1.23% based on BW gain and FCR, respectively. In contrast, the digestible lysine requirements of Korat chickens were higher than those recommended by NRC (1994), that is 1.10% total lysine. In comparison with slow-growing chickens in previous studies, the digestible lysine requirements of Korat chickens in the current study were also close to the digestible lysine requirements for Lohmann White based on BW gain, gain:feed ratio, and PD were 1.01 1.11 and 1.04%, respectively (Fatufe et al., 2004) and for New Hampshire x Columbian based on BW gain and gain:feed ratio were 1.01 and 1.21%, respectively (Han and Baker, 1991). It should be noted that digestible lysine levels determined in the present study are lower than those in the current guideline, which required 1.28% and 1.15% digestible lysine for Ross broilers in starter (0-10 days of age) and grower broilers (11-24 days of age), respectively (Aviagen, 2019). In comparison with guidelines of Cobb Broiler Management Guide (2015), the digestible lysine requirement of Korat chickens in the period from 1 to 21 days of age (1.08%) was also lower than that recommended for Cobb 500 in the period 0 to 10 days of age (1.18%) but it was slightly higher than that in the period from 11 to 22 days of age. Interestingly, the average optimum digestible lysine level (1.08%) for Korat chickens is between a range value reported by Faufe et al. (2004) and Han and Baker (1991) for slow-growing chickens as 1.05 and 1.11%, respectively. This is most likely because of the similar BW of birds at 21 days of age, which reported by Fatufe et al. (2004), the current study, and Han and Baker (1991) were 257 313 and 321g correspondings to the digestible lysine requirement in the diets as 1.05, 1.08, and 1.11%, respectively. In general, the digestible lysine requirements of Korat chickens were quite high. This statement is expressed clearly when the lysine requirements of Korat chickens were calculated as lysine intake per BWG, i.e., 17.3 mg/g. This value was 11.6% higher than the value in NRC (1994) although the value in NRC (1994) is recommended based on total lysine level. The reason why Korat chickens required high lysine intake/ BWG may be due to higher FCR, more detail, the lower utilization efficiency of protein and amino acid that has been proven by Tran et al. (2021). Another reason, the requirements of lysine intake/ BWG for Korat chickens are high as a result of their low feed consumption. As such, it must increase the concentration of nutrients in diets to satisfy the demand of Korat chickens.

Table 3.9 Protein and anino acid deposition of Korat chickens fed various digestible lysine concentrations from 1 to 21 days of age. Unless otherwise indicated, values are expressed as g/bird.

Item	Digestible lysine level					SEM	*P*-value
	0.87%	0.97%	1.07%	1.17%	1.27%		
Protein	40.16^{b}	45.99^{ab}	51.18^{a}	52.99^{a}	52.03^{a}	1.221	0.001
Lys	2.08^{b}	2.46^{ab}	2.62^{a}	2.69^{a}	2.78^{a}	0.070	0.005
Met	0.67^{b}	0.80^{ab}	0.84^{ab}	0.87^{a}	0.88^{a}	0.023	0.011
Thr	1.35^{b}	1.57^{ab}	1.62^{ab}	1.74^{a}	1.75^{a}	0.044	0.016
Arg	2.33	2.47	2.50	2.76	2.73	0.088	0.516
Ile	1.14^{b}	1.42^{ab}	1.28^{ab}	1.51^{ab}	1.62^{a}	0.052	0.017
Val	1.33^{b}	1.62^{ab}	1.58^{ab}	1.73^{a}	1.83^{a}	0.050	0.013
Leu	2.09^{b}	2.44^{ab}	2.65^{a}	2.67^{a}	2.71^{a}	0.066	0.008
His	1.35^{b}	1.56^{ab}	1.83^{a}	1.72^{a}	1.74^{a}	0.045	0.001
Phe	1.35^{b}	1.62^{ab}	1.71^{ab}	1.78^{a}	1.81^{a}	0.049	0.010

$^{a, b}$ Means within each row with different superscripts are significantly different ($P \leq 0.05$).

SEM = standard error of the mean.

3.5.2 Period II (22-42 days of age)

Similar toperiod I, Korat chickensin period II significantly responded on final BW (P<0.001), BW gain (P<0.001), and FCR (P<0.001), and UA in plasma (P=0.018) as increasing dietary lysine levels from 0.80% to 1.20%, whereas FI was not significantly different (P=0.107) among experimental treatments (Table 3.10). No mortality of birds was found in this period. The result clearly showed that the Korat chickens fed diets low dietary lysine (0.80%) was insufficient amounts for growth rate but when dietary lysine levels excess for requirement (1.10 and 1.20%), its growth rate was not improved compared to at 1.00% lysine in the diet. Likewise, FCR of birds was improved significantly when fed on dietscontaining dietary lysine from moderate to high levels (1.00 1.10 and 1.20%) compared to low dietary lysine content (0.80%). The data of BW gain of Korat chickens in this period fully coincided with the findings by Kino and Okumura (1986), which indicated the deficiency in a single essential amino acid affected the growth of birds. The effect of lysine deficiency on BW gain could cause by reduced muscle weights in the Pectoralis major (around 65%) muscle compared to in Anterior latissimus dorsi (40.5 to 45%) and the Sartorius (30% to 40%) muscle (Tesseraud et al.,1996). Conversely, Tesseraud et al. (1996) found also only slight changes in the absolute rates of protein breakdown. Therefore, it can be speculated thatthe reduction in BW gain as well as PD, mainly related to lower protein synthesis.

In contrast to BW gain, UA in plasma reached the highest level when birds fed the low dietary lysine level (0.80%) and it higher significantly compared to UA of birds fed the diets containing 1.10 and 1.20% lysine. Similar to period I, all experimental diets in period II were also formulated to the constant of essential amino acid content except for lysine. Therefore, the utilization efficiency of these amino acids in the diet containing low lysine is limited. Consequently, birds fed diets containing inadequate lysine levels lead to higher UA than birds fed on diets containing adequate or exceed lysine requirements. This result was consistent with the observations by Miles and Featherston (1974) and Donsbough et al. (2010).

Table 3.10 Growth performance of Korat chickens fed different digestible lysine levels from 22 to 42 days of age. Unless otherwise indicated, values are expressed as g/bird.

Item	Digestible lysine level					SEM	*P*-value
	0.80%	**0.90%**	**1.00%**	**1.10%**	**1.20%**		
Body weight							
Initial	255.4	254.9	255.3	255.6	255.0	0.366	0.977
Final	650.7^{b}	672.8^{ab}	688.3^{a}	690.9^{a}	690.5^{a}	3.736	<0.001
Gain	395.4^{b}	418.0^{ab}	433.0^{a}	435.3^{a}	435.5^{a}	3.691	<0.001
Feed intake	1016.9	995.3	942.0	949.9	961.7	10.49	0.107
FCR, g/g	2.57^{a}	2.38^{ab}	2.18^{c}	2.18^{c}	2.21^{bc}	0.034	<0.001
Uric acid, mg%	5.52^{a}	4.75^{ab}	4.77^{ab}	4.27^{b}	4.07^{b}	0.154	0.018

[a, b,c] Means within each row with different superscripts are significantly different ($P \leq 0.05$).

BWG = body weight gain; FCR = feed converion ratio; SEM = Standard error of the mean.

The PD in the whole-body was significantly affected ($P<0.001$) by an increase in dietary lysine (Table 3.11). In particular, PD of birds fed diets containing lysine from moderate levels (1.00%) increased significantly in comparison with low lysine (0.80%). However, with the increase in dietary lysine levels from 1.10 to 1.20%, there was no improvement in PD. Likewise, therewere significant in amino acid deposition ($P<0.05$) among dietary lysine levels, with the exception for Arg deposition. These results were totally in line with the finding on BW gain that has already been discussed above . This finding also coincided with the reports by Fatufe et al (2004) and Siqueira et al (2013), which showed that increasing dietary lysine significantly improved the deposition of amino acids.

Table 3.11 Protein and amino acid deposition of Korat chickens fed various digestible lysine concentrations from 22 to 42 days of age. Unless otherwise indicated, values are expressed as g/bird.

Item	**Digestible lysine level**					**SEM**	***P*-value**
	0.80%	**0.90%**	**1.00%**	**1.10%**	**1.20%**		
Protein	83.43^{b}	92.31^{ab}	98.62^{a}	98.68^{a}	96.34^{a}	1.342	<0.001
Lys	4.01^{b}	4.53^{ab}	5.28^{a}	4.94^{a}	5.03^{a}	0.130	0.005
Met	1.36^{b}	1.64^{ab}	1.71^{ab}	1.72^{ab}	1.76^{a}	0.050	0.040
Thr	2.66^{b}	2.92^{ab}	3.25^{a}	3.17^{ab}	3.34^{a}	0.077	0.013
Arg	4.46	4.84	5.21	5.06	5.31	0.107	0.067
Ile	2.44^{b}	3.01^{ab}	3.17^{a}	3.18^{a}	3.25^{a}	0.095	0.025
Val	2.70^{b}	3.22^{ab}	3.55^{ab}	3.60^{a}	3.73^{a}	0.116	0.016
Leu	6.14^{b}	7.02^{ab}	8.28^{a}	7.56^{ab}	7.66^{ab}	0.217	0.009
His	2.54^{b}	2.75^{b}	3.52^{a}	3.34^{a}	3.52^{a}	0.102	<0.001
Phe	2.82^{b}	3.00^{ab}	3.57^{a}	3.55^{a}	3.65^{a}	0.093	0.007

$^{a, b}$ Means within each row with different superscripts are significantly different ($P \leq 0.05$).

SEM = Standard error of the mean.

Using the broken-line regression analysis, the estimated digestible lysine requirements of Korat chickens from 22 to 42 days of age in the current study were 0.97 1.00 0.95 0.96 and 1.00% based on BW gain, FCR, UA, PD, and LysD, respectively. The result observed in the present study was close to the requirement of commercial broilers as in previous reports. For example, Cemin et al. (2017) estimated the digestible lysine requirement for male Cobb 500 aged 28 to 42 days was 0.97%. Dozier et al. (2010) showed that the digestible lysine requirements of Ross x Ross TP16 broilers from 28 to 42 days of age were 0.99 and 1.05% for BW gain and FCR, respectively. In the same study, they also found the digestible lysine requirements for male Cobb×Cobb 700 broilers (from 28 to 42 d of age) were 0.97 and 1.01% for BW gain and FCR, respectively. However, the digestible lysine requirement of Korat chickens in this period was higher than that in the study by Han and Baker (1994), who found that the digestible lysine requirements of Ross × Ross from 22 to 43 days of age for BW gain were at 0.85% for males and 0.78% for females and the needs for FCR were at 0.89% for males and 0.85% for female. In contrast, the digestible lysine requirement of Korat chickens in this study was lower than the results of Bernal et al. (2014). Particularly, the digestible lysine requirements of Cobb 500 from 22 to 35 days of age for BW gain and FCR were at 1.05, and 1.07%, respectively. In comparison with the digestible lysine requirement of Ross broilers (Aviagen. 2019), that is 1.02% in the period from 25 to 39 days of age, dietary lysine levels determined in the present study are slightly lower than those in the current guidelines and literature, while the digestible lysine of Korat chicken in this period (22-42 days of age) is slightly higher than the guidelines of Cobb Broiler Management Guide (2015) recommended for Cobb 500, that is 0.95% digestible lysine. When requirements are expressed as lysine intake/BWG, the requirements of Korat chickens (21.8 mg/g) were much higher in most previous studies (20.0 mg/g in NRC (1994), 20.5 mg/g in Han and Baker (1991), 19.1 mg/g in Dozier et al. (2010), 17.6 mg/g in Bernal et al. (2014), and 17.2 mg/g in Cemin et al. (2017) although BW of Korat chickens is much lower than commercial broilers in those studies. The reason why Korat chickens need high lysine intake/BWG has been already presented in period I. Overall, several other factors influence the lysine requirements of chickens such as genetic potential, sex, age, type of diet, levels of other nutrients, environment, measurement criterion, and statistical model (Leclercq, 1998; Rodehutscord and Pack, 1999, Pesti et al., 2009, Cemin et al., 2017).

3.5.3 Period III (43 to 63 days of age)

Standardized digestible amino acid values in the basal diet fed Korat chickens from 43 to 63 and from 64 to 84 days of age are shown in Table 3.7. The result indicated that the standardized digestible values of Lys, Met, Cys, Thr, Arg, His, and Phe were close in the calculated true digestible values derived from the total amino acid analyzed from feedstuffs (corn, soybean meal, and corn gluten meal) with digestible coefficients reported by Ajinomoto Heartland LLC (2009) (Table 3.6), whereas the values of Ile, Val, and Leu were lower than those calculated values in Table 3.6. No mortality of birds was observed in this period. The Korat chickens in this period significantly responded (P<0.05) on final BW, BW gain, FCR, and plasma UAasincreasing lysine contents in the diets from 0.69% to 1.09%, whereas FI was not significantly different (P>0.05) among experimental treatments (Table 1.12).

Similar to in periods I and II, all experientialdiets provided to Korat chickens in this period were formulated to contain a similar ME concentration (from 3200 to 3204 kcal/kg). As a result, the FI of Korat chickens was unaffected by the lysine levels in diet. Body weight gain of Korat chickens increased significantly when fed diets containing lysine from 0.89 to 1.09% compared to 0.69% but BW gain remained relatively constant among treatments of 0.89 0.99 and 1.09% lysine. Likewise, FCR was significantly improved when Korat chickens were fed a diet containing digestible lysine of 0.89% compared to 0.69%, but no further improvement in FCR when dietary lysine content was increased to 1.09%. These results clearly demonstrated that the diet contains 0.69% digestible lysine was inadequate for the growth rate of Korat chickens. Since the decrease in BW gain, whereas FI was constant, resulted in an increase in FCR when Korat chickens received insufficient lysine in the diet. The findings in this period were totally in line with previous reports (Labadan et al., 2001; Corzo et al., 2006; Samadi and Frank Liebert, 2007; Bernal et al., 2014; Cemin et al., 2017), which indicated the negative effect of dietary lysine deficiency on BW gain and FCR of birds.

Table 3.12 Growth performance of Korat chickens fed different digestible lysine levels from 43 to 63 days of age. Unless otherwise indicated, values are expressed as g/bird.

Item	**Digestible lysine level**					**SEM**	***P*-value**
	0.69%	**0.79%**	**0.89%**	**0.99%**	**1.09%**		
Body weight							
Initial	677.4	678.9	680.3	681.7	675.3	2.718	0.963
Final	1152.1^{c}	1173.0^{bc}	1194.6^{ab}	1195.1^{ab}	1204.3^{a}	5.277	0.004
Gain	474.7^{c}	494.2^{bc}	514.3^{ab}	513.5^{ab}	528.9^{a}	4.795	0.001
Feed intake	1460.0	1485.7	1436.1	1436.0	1443.8	11.40	0.631
FCR, g/g	3.08^{a}	3.01^{a}	2.79^{b}	2.80^{b}	2.73^{b}	0.033	<0.001
Uric acid, mg%	3.75	3.63	2.97	3.53	2.82	0.120	0.031

^{a,b,c}Means within each row with different superscripts are significantly different ($P \leq 0.05$).

BWG = body weight gain; FCR = feed converion ratio; SEM = Standard error of the mean.

Likewise, increasing dietary lysine levels significantly improved (*P*< 0.05) the deposition of protein and individual amino acids such as Lys, Met, Thr, Arg, Ile, Val, Leu, His, and Phe (Table 3.13). The data clearly showed that a diet contains 0.69% digestible lysine was inadequate lysine content for synthesis protein. As a result, the deposition of protein and amino acid in the whole-body of Korat chickens decreased significantly compared to the diets containing from 0.89 to 1.09% lysine. The resuls of the protein and lysine deposition in the whole-body of Korat chickens are in line with the finding on BW gain. Similar results were reported by Fatufe et al. (2004) and Siqueira et al. (2013), indicating that a significant improvement in the protein/ amino acid accretion of birds fed with increasing lysine content. Needless to say, low lysine levels contain an insufficient amount to ensure the accretion of lysine/ protein in order to further achieve an optimum BW gain of Korat chickens. The decline in the deposition of lysine/ protein as well as growth rate associated with lysine deficient diets could due to a lower protein synthesis rate or a higher degradation rate, or a simultaneous alteration in both components of protein turnover in the whole-body (Roeder and Broderick, 1981; Muramatsu et al., 1986; Tesseraud et al., 1992, 1996; Urdaneta-Rincon and Leeson, 2004).

Using the broken-line regression analysis, digestible lysine requirements were estimated to be 0.93% for both BW gain and FCR, 0.89% for PD, and 0.90% for LysD (Table 3.16). However, the UA in plasma cannot be estimated because the data did not conform to the regression model. The optimum digestible lysine levels of Korat chickens in the period from 43 to 63 days of age was close to those in Cobb broiler management guide (2015) from 43 to market, namely 0.90% digestible lysine but it was lower than that of Ross in the period from 40 days of age to market in the current

Table 3.13 Protein and anino acid deposition of Korat chickens fed various digestible lysine concentrations from 43 to 63 days of age. Unless otherwise indicated, values are expressed as g/bird.

Item	**Digestible lysine level**					**SEM**	***P*-value**
	0.69%	**0.79%**	**0.89%**	**0.99%**	**1.09%**		
Protein	93.47^{c}	101.6^{bc}	108.6^{ab}	108.0^{ab}	112.4^{a}	1.504	<0.001
Lys	5.19^{b}	5.71^{ab}	6.23^{a}	6.27^{a}	6.29^{a}	0.115	<0.001
Met	2.00^{b}	2.21^{ab}	2.42^{ab}	2.46^{a}	2.48^{a}	0.059	0.017
Thr	3.74^{b}	4.11^{ab}	4.35^{a}	4.29^{a}	4.48^{a}	0.077	0.007
Arg	5.36^{c}	5.93^{bc}	6.51^{ab}	6.63^{ab}	6.95^{a}	0.157	0.001
Ile	2.66^{b}	4.04^{ab}	4.30^{a}	4.20^{a}	4.38^{a}	0.068	<0.001
Val	3.35^{b}	3.81^{ab}	4.13^{a}	4.05^{a}	4.22^{a}	0.087	0.001
Leu	5.51^{b}	6.01^{ab}	6.48^{a}	6.50^{a}	6.53^{a}	0.114	0.003
His	2.96^{b}	4.23^{ab}	4.36^{ab}	4.32^{ab}	4.48^{a}	0.059	0.030
Phe	3.65^{b}	3.99^{ab}	4.29^{a}	4.30^{a}	4.39^{a}	0.082	0.007

[a, b,c] Means within each row with different superscripts are significantly different ($P \leq 0.05$).
SEM = Standard error of the mean.

guidelines (Aviagen, 2019), that is 0.96% digestible lysine. Nevertheless, the digestible lysine requirement of Korat chickens in the current study was higher than those of commercial broilers of the same age in other previous reports. For example, Labadan et al. (2001) reported that Ross male × Avian female from 5 to 8 wk of age required 0.75% digestible lysine. In addition, Corzo et al. (2006) determined the digestible lysine of Hubbard Ultra Yield males from 42 to 56 days of age at 0.85%. The total lysine requirement of Cobb 500 from 50 to 65 days of age was also estimated at 0.95% (Samadi and Frank Liebert, 2007). Generally, the digestible lysine requirements of Korat chickens in this period were also quite high when they are expressed as lysine intake/BWG. Its value was higher at least 9% to 43.9% in comparison with previous studies (NRC, 1994; Labadan et al., 2001; Corzo et al., 2006). The lysine requirement of Korat chickens was higher than commercial broilers which is likely due to environmental conditions. In particular, the Korat chickens in the current study were raised in an open-sided and naturally ventilated barn in the tropical climate. It was known that high ambient temperature caused a reduction in feed intake (Han and Baker, 1993) and therefore required a higher lysine content in the diet.

3.5.4 Period IV (64 to 84 days of age)

The mortality was 0.0% in the period 64-84 days of age. The response of Korat chickens in this period to dietary lysine on growth performance and plasma UA are shown in Table 3.14. The results showed similar trends to those in the previous periods. In particular, the final BW, BW gain, FCR, and plasma UA were influenced by dietary treatments (P<0.05), whereas FI had no significant difference among treatments. As the dietary lysine increased from 0.69% to 0.89%, the BW gain increased significantly but there were no significant differences in BW gain of birds fed diets of 0.89% 0.99% and 1.09% lysine. In contrast, values on the FCR improved significantly with increasing dietary lysine contents. Particularly, FCR decreased significantly when birds fed on 0.99% lysine level compared to 0.69% Lys level in the diet. However, there have no significant differences in FCR of birds fed on the diet containing higher dietary lysine. Similarly, plasma UA in Korat chickens was significantly affected by dietary lysine. Birds fed with the highest lysine level had the lowest UA in plasma but it is no significant difference when dietary lysine content increase from 0.79% to 1.09%. These findings were in agreement with studies of Labadan et al. (2001), Corzo et al. (2006), Samadi and Frank Liebert (2007), Bernal et al. (2014), Cemin et al. (2017), who found that insufficient lysine content in the diets caused negative impacts on BW gain and FCR of due to a lower protein synthesis rate or a higher degradation rate, or a simultaneous alteration in both components of protein turnover in the whole-body (Roeder and Broderick, 1981; Muramatsu et al., 1986; Tesseraud et al., 1992, 1996; Urdaneta-Rincon and Leeson, 2004).

Table 3.14 Growth performance of Korat chickens fed different digestible lysine levels from 64 to 84 days of age. Unless otherwise indicated, values are expressed as g/bird.

Item	Digestible lysine level					SEM	*P*-value
	0.69%	**0.79%**	**0.89%**	**0.99%**	**1.09%**		
Body weight							
Initial	1,259	1,259	1,259	1,264	1,262	2.614	0.939
Final	1,753[b]	1,789[ab]	1,801[a]	1,807[a]	1,802[a]	5.817	0.011
Gain	494.5[b]	529.8[ab]	542.6[a]	542.4[a]	540.2[a]	5.077	0.004
Feed intake	1,712	1,750	1,745	1,713	1,699	16.23	0.840
FCR, g/g	3.46[a]	3.31[ab]	3.22[ab]	3.16[b]	3.15[b]	0.033	0.006
Uric acid, mg%	5.22[a]	4.74[ab]	4.55[ab]	4.78[ab]	3.98[b]	0.126	0.025

[a, b]Means within each row with different superscripts are significantly different ($P \leq 0.05$).

BWG = body weight gain; FCR = feed converion ratio; SEM = Standard error of the mean.

The response of Korat chickens from 64 to 84 days of age to dietary lysine on the deposition of protein and amino acids in the whole-body is shown in Table 3.15. There had a significant increase of the PD when birds fed on 0.79% lysine compared to 0.69% but there had no significant differences when the dietary lysine increase from 0.79% to 1.09%. The deposition of Lys, Thr, Arg, Val, Leu, His, and Phe increased significantly when birds fed on 0.89% lysine compared to 0.69% but there had no significant differences when the dietary lysine increase from 0.89% to 1.09%. The deposition of Met increased significantly to increasing dietary lysine levels from 0.69% to 1.09% but there had no significant differences from 0.79 to 1.09% lysine. The deposition of Ile increased significantly from 0.69% to 0.99% lysine; however, there had also no significant difference from 0.79% to 1.09% Lys. In general, the significant increase of the PD and amino acid deposition was maybe due to an increase in the protein synthesis rate (Salter et al., 1990).

Table 3.15 Protein and anino acid deposition of Korat chickens fed different digestible lysine levels from 64 to 84 days of age. Unless otherwise indicated, values are expressed as g/bird.

Item	**Digestible lysine level**					**SEM**	***P*-value**
	0.69%	**0.79%**	**0.89%**	**0.99%**	**1.09%**		
Protein	99.57^{b}	111.8^{a}	118.1^{a}	118.5^{a}	117.6^{a}	1.634	<0.001
Lys	5.41^{b}	6.17^{ab}	6.70^{a}	6.48^{a}	6.64^{a}	0.139	0.005
Met	2.35^{b}	2.49^{ab}	2.60^{ab}	2.62^{ab}	2.69^{a}	0.041	0.050
Thr	3.80^{b}	4.23^{ab}	4.43^{a}	4.45^{a}	4.51^{a}	0.073	0.001
Arg	6.00^{b}	6.63^{ab}	7.25^{a}	7.32^{a}	7.34^{a}	0.161	0.011
Ile	3.72^{b}	4.27^{ab}	4.41^{ab}	4.50^{a}	4.58^{a}	0.096	0.015
Val	3.85^{b}	4.32^{ab}	4.49^{a}	4.52^{a}	4.59^{a}	0.081	0.010
Leu	5.94^{b}	6.48^{ab}	6.96^{a}	7.01^{a}	7.15^{a}	0.134	0.008
His	4.06^{b}	4.47^{ab}	4.85^{a}	4.76^{a}	4.80^{a}	0.088	0.006
Phe	4.07^{b}	4.38^{ab}	4.61^{a}	4.60^{a}	4.64^{a}	0.065	0.009

$^{a,\,b}$Means within each row with different superscripts are significantly different ($P \leq 0.05$).

SEM = Standard error of the mean.

The estimated digestible lysine requirements of Korat chickens in the period 64-84 d of age were 0.82 0.87 0.88 0.84 and 0.85%, respectively, for optimum BW gain, FCR, UA, PD, and LysD based on broken-line regression analysis (Table 3.16). Similar to period II, the digestible Lys requirement of Korat chicken in this period was higher for feed conversion than BW gain. The optimal dietary lysine level for BW gain was very close to PD. This finding was totally in agreement with Fatufe et al. (2004). Similar to the results of the previous periods, the requirements of Korat chickens in this period were also quite high. It has been well-document that, the lysine requirements of chickens decrease with increasing age. Although it has no information of lysine requirements of commercial broilers from 9 to 12 weeks of age, the lysine requirements of Korat chickens from 9 to 12 weeks of age were higher than those recommended for commercial broilers from 5 or 6 to 8 weeks of age.

Overall, the lysine requirement of Korat chickens decreased with age. This observation was proportionate to the reduction in the protein requirement of Korat chickens, which was conducted by Maliwan et al. (2019). This finding was also in line with previous reports, which indicated the requirement for most essential amino acids decreases with the increasing age of the growing animal (Graber et al., 1971; NRC, 1994; Labadan et al., 2001; Bernal et al., 2014; Cemin et al., 2017). Differences in the amino acid need during the different growing periods are due to differences in percentage growth rate and the requirements for tissue maintenance (Mitchell, 1959).

Table 3.16 The optimum digestible lysine levels of Korat chickens in the period I, II, III, and IV estimated by the linear broken-line model.

Item	Equation[1]	Estimated requirement	*P*-value	R^2
Period 1				
BW gain	$Y = 272.0 - 210.1 \times (1.08 - x)$	1.08	0.0020	0.307
FCR	$Y = 1.58 + 2.47 \times (1.03 - x)$	1.03	<0.0001	0.565
UA	$Y = 4.24 + 14.30\ (1.12 - x)$	1.12	<0.0001	0.649
PD	$Y = 52.51 - 55.09 \times (1.09 - x)$	1.09	<0.0001	0.437
LysD	$Y = 2.74 - 2.73 \times (1.10 - x)$	1.10	0.0006	0.384
Period II				
BW gain	$Y = 434.6 - 226.0 \times (0.97 - x)$	0.97	<0.0001	0.483
FCR	$Y = 2.19 + 1.90 \times (1.00 - x)$	1.00	<0.0001	0.499
UA	$Y = 4.37 + 7.67 \times (0.95 - x)$	0.95	0.0008	0.333
PD	$Y = 97.88 - 88.77 \times (0.96 - x)$	0.96	0.0002	0.397
LysD	$Y = 5.07 - 5.27 \times (1.00 - x)$	1.00	0.0007	0.370
Period III				
BW gain	$Y = 521.2 - 198.0 \times (0.925 - x)$	0.93	0.0001	0.4899
FCR	$Y = 2.765 + 1.425 \times (0.926 - x)$	0.93	<0.0001	0.5080
UA	NE[2]			
PD	$Y = 109.7 - 81.12 \times (0.8897 - x)$	0.89	<0.0001	0.5967
LyD	$Y = 6.279 - 5.225 \times (0.899 - x)$	0.90	<0.0001	0.6051
Period IV				
BW gain	$Y = 541.7 - 353.2 \times (0.824 - x)$	0.82	0.0003	0.2900
FCR	$Y = 3.174 + 1.550 \times (0.874 - x)$	0.87	0.0008	0.3782
UA	$Y = 4.328 + 4.767 \times (0.877 - x)$	0.88	0.0167	0.3196
PD	$Y = 118.1 - 121.9 \times (0.842 - x)$	0.84	<00001	0.4754
LysD	$Y = 6.608 - 7.575 \times (0.848 - x)$	0.85	0.0005	0.4161

[1]The linear broken-line model was fitted as $Y = L + U \times (R - x)$, where Y is the dependent variable; x is the dietary lysine level as independent variable; R is the optimum response of dietary lysine; L is the response at x = r; and U = the steepness of the curve. In this model, Y = L when x > R.

[2]NE = Not estimated because data did not conform to the regression model.

BW = body weight; FCR = feed conversion ratio; UA = uric acid; PD = protein deposition; LysD = lysine deposition.

3.6 Conclusion

Through the present results, digestible lysine levels of 1.08 0.98 0.91 and 0.85% or 230 471 639 and 776 mg/bird/d were recommended for Korat chickens in periods I (from 1 to 21 days of age), II (from 22 to 42 days of age), III (from 43 to 63 days of age), and IV (from 64 to 84 days of age), respectively, based on averages of the measured criteria.

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CHAPTER IV

ESTIMATION OF SULFUR AMINO ACID REQUIREMENTS FOR KORAT CHICKENS FROM 0 TO 12 WEEKS OF AGE

4.1 Abstract

The current study was conducted to estimate the digestible sulfur amino acid (SAA) requirement for Korat chickens from 1 to 84 days of age. The study consisted of four age periods (I: 1-21 d; II: 22-42 d; III: 43-63 d; IV: 64-84 d). Korat chickens in each period were randomly allotted to 5 dietary treatments with 6 replicates (16 birds per unit/pen) in a completely randomized design. The birds were fed five levels of digestible SAA: 0.63 0.73 0.83 0.93 and 1.03% in period I; 0.58 0.66 0.74 0.82 and 0.90% in period II; 0.50 0.58 0.66 0.74 and 0.82% in both periods III and IV. Standardized amino acid digestibility assays were carried out with Korat chickens to determine the digestibility of amino acids in the basal diets. The standardized SAA digestibility of the basal diets was established at 0.63 0.59 and 0.52% in periods I, II, and both III and IV, respectively. The SAA requirements were estimated using the broken-line regression analysis. The results indicated that the Korat chickens significantly (P<0.05) responded to dietary SAA levels in body weight (BW) gain, feed conversion ratio (FCR), uric acid (UA) in plasma (except in period II and III), protein deposition (PD), and amino acid deposition in all periods. The estimates of

digestible SAA requirements for Korat chickens were 0.83 0.81 0.85 0.85 and 0.82% for BW gain, FCR, UA, PD, and sulfur amino acid deposition (SAAD), respectively, in period I; 0.74 0.76 0.75 and 0.74% for BW gain, FCR, PD, and SAAD, respectively, in period II; 0.67 0.66 0.67 and 0.68% for BW gain, FCR, PD, and SAAD, respectively, in period III; and 0.65 0.66 0.66 and 0.67% for BW gain, FCR, PD, and SAAD, respectively, in period IV. In brief, the digestible SAA requirements for Korat chickens were 0.83 0.75 0.67 and 0.66% in periods I, II, III, and IV, respectively, based upon averages of measured criteria.

Key words: Korat chicken, sulfur amino acid, growth performance

4.2 Introduction

Similar to other amino acids, Met and Cys are the components of tissue proteins and thus serve as substrates for protein synthesis. In addition, Met is also involved in the metabolism of methyl group and in the synthesis of other SAA, especially Cys. Methionine can be converted irreversibly to Cys via transsulfuration. Cys is required for the synthesis of glutathione and taurine, which are essential compounds for host defense against oxidative stress. Therefore, Cys can be considered as a nonessential amino acid. For this reason, the requirements of these amino acids are generally considered together as requirements for Met plus Cys. (Garcia and Batal, 2005).

Methionine is an essential amino acid which plays a crucial role in the growth of poultry and is the first limiting amino acid in common poultry diets. Proper supplementation of Met or SAA to a diet deficient in Met and Cys improved growth performance and carcass quality of fast-growing broilers has been well documented previously (Jensen et al., 1989; Hickling et al., 1990; Moran, 1994; Schutte and Pack, 1995; Esteve-Garcia and Llaurado, 1997; Wallis, 1999; Esteve-Garcia and Mack, 2000; Lemme et al., 2002; Vieira et al., 2004; Garcia and Batal, 2005; Lumpkins et al., 2007; Goulart et al., 2011; Conde-Aguilera et al., 2013). However, SAA above the requirements have no further improvement, in addition this may have a negative impact on growth performance. Therefore, this has an economic impact, it will lead to an unnecessary additional feed cost. Furthermore, the excess supply of SAA could affect the environment as nitrogen excretion from chickens increases (Belloir et al., 2017).

It is noted that the sulfur amino acid requirements are influenced by genetic potential, dietary protein concentration, measurement criteria, age, and environment (Graber et al., 1971; Boomgaardtd and Baker, 1973; Mendonca and Jensen, 1989; Han and Baker, 1993; NRC, 1994; Vieira et al., 2004; Lumpkins et al., 2007; Goulart et al., 2011; Faridi et al., 2016; Millecam et al., 2021). According to the NRC (1994) recommendation, the SAA requirement (based on total content) for fast-growing broilers in periods 0-3 3-6 and 6-8 wk of age were 0.90 0.72 and 0.60%, respectively. In addition, digestible SAA requirements for Cobb were reported as 0.87 0.76 0.75 and 0.66% in periods 1-7 8-21 22-35 and 36-42 days of age, respectively (Goulart et al., 2011). The optimal digestible of SAA level was 0.69 0.66 and 0.62% for Ross308 male in the starter (0-10 d), grower (11-23 d), and finisher (24-35) phase, respectively (Millecam et al., 2021). However, the reports related to dietary SAA study in slow-growing broilers are very scarce.

4.3 Objective

Due to the obvious reasons above, the current study was conducted objective of estimating the optimum levels of SAA for Korat chickens based on growth performance, UA in plasma, PD, and SAAD in the whole-body.

4.4 Materials and methods

4.4.1 Ethical considerations

As described in chapter III (see section 3.4.1).

4.4.2 Animals, housing and experimental design

The experiment was divided into 4 periods as 0-3 3-6 6-9 and 9-12 wk of age to estimate the sulfur amino acid requirements of Korat chickens. A total of 2,092 Korat chickens were used in the current study including 1,920 birds in dose-response, 64 birds in the comparative slaughter technique, and 108 birds in the determination of digestion. The birds were obtained from a hatchery belonging to SUT, Nakhon Ratchasima, Thailand. As the gender of the Korat chickens cannot be ascertained one day after hatch, it was decided to use only un-sexed birds in the first period (0-3 wk of age), whereas mixed-sex Korat chickens were used in the other periods (3-6 6-9 and 9-12 wk of age). The experimental birds were vaccinated against Marek’s disease (FATRO S.p.A., Bologna, Italy) at the hatchery. On days 7 and 21, all birds were inoculated with Newcastle disease and infectious bronchitis vaccines (FATRO S.p.A., Bologna, Italy). On day 14, the birds were vaccinated against infectious bursal disease vaccine (FATRO S.p.A., Bologna, Italy). On day 28, they were inoculated with the flow pox vaccine (FATRO S.p.A., Bologna, Italy). The experimental birds were raised under the management as described in chapter III (see section 3.4.2).

In the first period (0-3 wk of age), a total of 544 hatchlings were used in this period. Sixteen hatchlings were killed on arrival to determine the initial body composition and 480 birds were randomly allocated to 5 dietary treatments (Table 4.1) with 6 replicates of 16 birds per pen. The remaining birds were raised on the floor pen up to 12 days of age and fed on a diet that contained 0.83% SAA (Table 4.1). On day 13, birds were randomly allocated to metabolic cages with 6 replicates of 8 birds per cage. The birds still were fed a diet containing 0.83% SAA until day 15. On day 16, the birds were fed a diet containing 0.63% SAA (Table 4.1) until day 20.

In the second period (3-6 wk of age), a total of 532 hatchlings were initially fed a diet containing 2980 kcal ME/kg and 21.26% crude protein (as fed) from hatch to 21 d of age. On day 22, sixteen birds were used to determine the initial body composition and 480 mixed-sex birds (male and female are equal) were randomly allocated to 5 dietary treatments (Table 4.3) with 6 replicates of 16 birds per pen. The remaining birds were fed on a diet that containing 0.74% SAA (Table 4.3) up to the age of 33 days. On day 34, birds were randomly distributed to metabolic cages with 6 replicates of 6 birds per unit. The birds still were fed a diet containing 0.74% SAA. On day 37, the birds were a diet containing 0.58% SAA (Table 4.3) until day 41.

In the third period (6-9 wk of age), a total of 520 hatchlings were initially fed a diet containing 2,980 kcal ME/kg and 21.26% crude protein (as fed) from hatch to 21 d of age and a diet containing 3,150 kcal ME/kg and 20.45% crude protein from 22 to 42 d of age. On day 43, sixteen birds were used to determine the initial body composition and 480 mixed-sex birds were randomly allocated to 5 dietary treatments (Table 4.5) with 6 replicates of 16 birds per pen. The remaining birds were fed on a diet containing 0.66% SAA (Table 4.5) until 54 days of age. After that one day, 24 mixed-sex birds were randomly allocated to 6 metabolic cages with 4 birds per unit. The birds still were fed a diet containing 0.66% SAA. On day 58, the birds were fed a diet containing 0.50% SAA (Table 4.5) until day 62.

In the final period (9-12 wk of age), a total of 496 hatchlings were initially fed a diet containing 2,980 kcal ME/kg and 21.26% crude protein (as fed) from hatch up to 21 d of age, following with a diet containing 3,150 kcal ME/kg and 20.45% crude protein up to 42 d of age. Then, the birds were fed a diet containing 3,200 kcal ME/kg and 18.00% crude protein up to 63 d of age. On day 64, sixteen birds were used to determine the initial body composition and 480 mixed-sex birds were randomly allocated to 5 dietary treatments (Table 4.5) with 6 replicates of 16 birds per pen.

4.4.3 Experimental diets

There were five dietary SAA levels in each trial period. Particularly, 0.63 0.73 0.83 0.93 and 1.03% SAA in the period 0-3 wk of age, 0.58 0.66 0.74 0.82 and 0.90% SAA in the period 3-6 wk of age, and 0.50 0.58 0.66 0.74 and 0.82% SAA in the both periods 6-9 wk and 9-12 wk of age. Ingredients and chemical composition of diets are presented in Table 4.1, Table 4.2, Table 4.3, Table 4.4, Table 4.5, and Table 4.6. The experimental diets were formulated to meet the requirements recommended by Maliwan (2018, 2019) regarding metabolizable energy and crude protein contents. Other dietary nutrient compositions were formulated to meet or exceed the nutrient requirements recommended by the NRC (1994), with the exception of the SAA level. L-glutamic acid was used to replace variable levels of SAA to maintain isonitrogenous and isocaloric diets. Feedstuffs were analyzed for crude protein and total amino acid contents prior feed formulation. The value of digestible amino acids in feedstuffs was determined using digestible coefficients (Ajinomoto Heartland LLC, 2009) and analyzed the total amino acid contents of the ingredients. The diets were fed in a mash form.

4.4.4 Data and sample collection

As described in chapter III (see section 3.4.4).

4.4.5 Chemical analysis

As described in chapter III (see section 3.4.5).

4.4.6 Statistical analysis

As described in chapter III (see section 3.4.5).

Table 4.1 The ingredient of the experimental diets fed to Korat chickens in period I (1-21 days of age).

	Level of dietary sulfur amino acids				
Item	**0.63%**	**0.73%**	**0.83%**	**0.93%**	**1.03%**
Ingredient, %					
Corn	59.55	59.55	59.55	59.55	59.55
Soybean meal, 44% CP	29.85	29.85	29.85	29.85	29.85
Corn gluten meal	3.00	3.00	3.00	3.00	3.00
Rice bran oil	0.44	0.44	0.44	0.44	0.44
Calcium carbonate	1.68	1.68	1.68	1.68	1.68
Monocalcium phosphate	1.63	1.63	1.63	1.63	1.63
Sodium chloride	0.44	0.44	0.44	0.44	0.44
Premix[1]	0.50	0.50	0.50	0.50	0.50
Glutamic acid, purity 99%	0.40	0.30	0.20	0.10	0.00
Cornstarch	2.15	2.15	2.15	2.14	2.14
DL-Met, purity 99%	0.00	0.10	0.20	0.31	0.41
L-Lys HCl, purity 78%	0.17	0.17	0.17	0.17	0.17
L-Thr, purity 98.5%	0.17	0.17	0.17	0.17	0.17
L-Arg, purity 99%	0.02	0.02	0.02	0.02	0.02
Total, %	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

[1]Premix contained the following nutrients (units are expressed per kg of diet): vitamin A, 15,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin K3, 5 mg; vitamin B1, 2 mg; vitamin B2, 7 mg; vitamin B6, 4 mg; vitamin B12, 25 mg; pantothenic acid, 11.04 mg; nicotinic acid, 35 mg; folic acid, 1 mg; biotin, 15 µg; choline chloride, 250 mg; Cu, 1.6 mg; Mn, 60 mg; Zn, 45 mg; Fe, 80 mg; I, 0.4 mg; Se, 0.15 mg.

Table 4.2 Chemical composition of the diets fed to Korat chickens in period I (1-21 days of age). Unless otherwise indicated, values are expressed as % as fed.

	Level of dietary sulfur amino acids				
Item	**0.63%**	**0.73%**	**0.83%**	**0.93%**	**1.03%**
Calculated composition[1]					
ME, kcal/kg	2980	2981	2981	2982	2983
Crude protein	21.26	21.26	21.26	21.26	21.26
Digestible Lys	1.09	1.09	1.09	1.09	1.09
Digestible Met	0.31	0.41	0.51	0.61	0.71
Digestible Met + Cys	0.63	0.73	0.83	0.93	1.03
Digestible Thr	0.80	0.80	0.80	0.80	0.80
Digestible Arg	1.24	1.24	1.24	1.24	1.24
Digestible Ile	0.82	0.82	0.82	0.82	0.82
Digestible Val	0.97	0.97	0.97	0.97	0.97
Digestible Leu	1.83	1.83	1.83	1.83	1.83
Digestible His	0.63	0.63	0.63	0.63	0.63
Digestible Phe	0.95	0.95	0.95	0.95	0.95
Digestible Trp	0.20	0.20	0.20	0.20	0.20
Ca	1.02	1.02	1.02	1.02	1.02
Available P	0.46	0.46	0.46	0.46	0.46
Analyzed composition					
Dry matter	89.64	89.54	89.59	90.08	89.47
Crude protein	21.12	21.38	21.36	21.33	21.44
Crude fiber	2.39	2.37	2.34	2.46	2.41
Ether extract	3.55	3.54	3.60	3.43	3.42
Crude ash	5.98	6.09	6.10	6.08	6.12

[1]The values were calculated according to the values of feedstuffs (NRC 1994) except for crude protein and amino acids; CP was calculated from analyzed CP contents of feedstuffs; digestible amino acids were calculated by digestible coefficients (Ajinomoto Heartland LLC, 2009) and analyzed amino acid contents of the ingredients plus the values of synthetic amino acids (digestible coefficients = 100%).

4.4.7 Experimental location

The experiment was conducted at Suranaree University of Technology's poultry farm, the Center for Scientific and Technological Equipment Building 10, Suranaree University of Technology.

4.4.8 Ethical considerations

The experiment was done from February 2019 to May 2019.

Table 4.3 The ingredient of the experimental diets fed to Korat chickens in period II (22-42 days of age).

	Level of dietary sulfur amino acids				
Item	**0.58%**	**0.66%**	**0.74%**	**0.82%**	**0.90%**
Ingredient, %					
Corn	59.50	59.50	59.50	59.50	59.50
Soybean meal, 44% CP	31.70	31.70	31.70	31.70	31.70
Rice bran oil	3.70	3.70	3.70	3.70	3.70
Calcium carbonate	1.60	1.60	1.60	1.60	1.60
Monocalcium phosphate	1.10	1.10	1.10	1.10	1.10
Sodium chloride	0.47	0.47	0.47	0.47	0.47
Premix[1]	0.50	0.50	0.50	0.50	0.50
Glutamic acid, purity 99%	0.96	0.88	0.80	0.72	0.64
Cornstarch	0.27	0.27	0.27	0.26	0.26
DL-Met, purity 99%	0.00	0.08	0.16	0.25	0.33
L-Lys HCl, purity 78%	0.06	0.06	0.06	0.06	0.06
L-Thr, purity 98.5%	0.14	0.14	0.14	0.14	0.14
Total, %	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

[1]Premix contained the following nutrients (units are expressed per kg of diet): vitamin A, 15,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin K3, 5 mg; vitamin B1, 2 mg; vitamin B2, 7 mg; vitamin B6, 4 mg; vitamin B12, 25 mg; pantothenic acid, 11.04 mg; nicotinic acid, 35 mg; folic acid, 1 mg; biotin, 15 µg; choline chloride, 250 mg; Cu, 1.6 mg; Mn, 60 mg; Zn, 45 mg; Fe, 80 mg; I, 0.4 mg; Se, 0.15 mg.

Table 4.4 Chemical composition of the diets fed to Korat chickens in period II (22-42 days of age). Unless otherwise indicated, values are expressed as % as fed.

	Level of dietary sulfur amino acids				
Item	**0.58%**	**0.66%**	**0.74%**	**0.82%**	**0.90%**
Caculated composition[1]					
ME, kcal/kg	3150	3151	3151	3152	3153
Crude protein	20.45	20.45	20.45	20.45	20.45
Digestible Lys	1.02	1.02	1.02	1.02	1.02
Digestible Met	0.28	0.36	0.44	0.53	0.61
Digestible Met + Cys	0.58	0.66	0.74	0.82	0.90
Digestible Thr	0.75	0.75	0.75	0.75	0.75
Digestible Arg	1.23	1.23	1.23	1.23	1.23
Digestible Ile	0.79	0.79	0.79	0.79	0.79
Digestible Val	0.93	0.93	0.93	0.93	0.93
Digestible Leu	1.60	1.60	1.60	1.60	1.60
Digestible His	0.61	0.61	0.61	0.61	0.61
Digestible Phe	0.88	0.88	0.88	0.88	0.88
Digestible Trp	0.21	0.21	0.21	0.21	0.21
Ca	0.90	0.90	0.90	0.90	0.90
Available P	0.35	0.35	0.35	0.35	0.35
Analyzed composition					
Dry matter	89.89	89.74	90.06	89.67	89.90
Crude protein	20.30	20.24	20.36	20.27	20.33
Crude fiber	2.59	2.64	2.61	2.67	2.60
Ether extract	6.48	6.71	6.64	6.57	6.53
Crude ash	5.67	5.58	5.68	5.74	5.66

[1]The values were calculated according to the values of feedstuffs (NRC 1994) except for crude protein and amino acids; CP was calculated from analyzed CP contents of feedstuffs; digestible amino acids were calculated by digestible coefficients (Ajinomoto Heartland LLC, 2009) and analyzed amino acid contents of the ingredients plus the values of synthetic amino acids (digestible coefficients = 100%).

Table 4.5 The ingredient of the diets fed to Korat chickens in period III (43-63 days of age) and period IV (64-84 days of age).

	Level of dietary sulfur amino acids				
Item	**0.50%**	**0.58%**	**0.66%**	**0.74%**	**0.82%**
Ingredient, %					
Corn	65.00	65.00	65.00	65.00	65.00
Soybean meal, 44% CP	24.13	24.13	24.13	24.13	24.13
Rice bran oil	3.35	3.35	3.35	3.35	3.35
Calcium carbonate	1.48	1.48	1.48	1.48	1.48
Monocalcium phosphate	0.98	0.98	0.98	0.98	0.98
Sodium chloride	0.47	0.47	0.47	0.47	0.47
Premix[1]	0.50	0.50	0.50	0.50	0.50
Glutamic acid, purity 99%	1.40	1.32	1.24	1.16	1.08
Cornstarch	2.16	2.16	2.16	2.16	2.16
DL-Met, purity 99%	0.00	0.08	0.16	0.24	0.32
L-Lys HCl, purity 78%	0.18	0.18	0.18	0.18	0.18
L-Thr, purity 98.5%	0.23	0.23	0.23	0.23	0.23
L-Arg, purity 99%	0.08	0.08	0.08	0.08	0.08
L-Ile, purity 99%	0.03	0.03	0.03	0.03	0.03
L-Trp, purity 98.5%	0.01	0.01	0.01	0.01	0.01
Total, %	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

[1]Premix contained the following nutrients (units are expressed per kg of diet): vitamin A, 15,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin K3, 5 mg; vitamin B1, 2 mg; vitamin B2, 7 mg; vitamin B6, 4 mg; vitamin B12, 25 mg; pantothenic acid, 11.04 mg; nicotinic acid, 35 mg; folic acid, 1 mg; biotin, 15 µg; choline chloride, 250 mg; Cu, 1.6 mg; Mn, 60 mg; Zn, 45 mg; Fe, 80 mg; I, 0.4 mg; Se, 0.15 mg.

Table 4.6 Chemical composition of the diets fed to Korat chickens in period III (43-63 days of age) and period IV (64-84 days of age). Unless otherwise indicated, values are expressed as % as fed.

	Level of dietary sulfur amino acids				
Item	**0.50%**	**0.58%**	**0.66%**	**0.74%**	**0.82%**
Calculated composition[1]					
ME, kcal/kg	3200	3200	3201	3201	3202
Crude protein	18.00	18.00	18.00	18.00	18.00
Digestible Lys	0.93	0.93	0.93	0.93	0.93
Digestible Met	0.25	0.33	0.41	0.49	0.57
Digestible Met + Cys	0.50	0.58	0.66	0.74	0.82
Digestible Thr	0.74	0.74	0.74	0.74	0.74
Digestible Arg	1.09	1.09	1.09	1.09	1.09
Digestible Ile	0.68	0.68	0.68	0.68	0.68
Digestible Val	0.79	0.79	0.79	0.79	0.79
Digestible Leu	1.42	1.42	1.42	1.42	1.42
Digestible His	0.42	0.42	0.42	0.42	0.42
Digestible Phe	0.74	0.74	0.74	0.74	0.74
Digestible Trp	0.18	0.18	0.18	0.18	0.18
Ca	0.82	0.82	0.82	0.82	0.82
Available P	0.31	0.31	0.31	0.31	0.31
Analyzed composition					
Dry matter	90.23	90.38	90.26	90.42	90.44
Crude protein	17.93	17.95	17.96	18.04	17.89
Crude fiber	2.29	2.32	2.24	2.33	2.26
Ether extract	6.13	6.07	6.02	6.06	6.14
Crude ash	5.08	5.00	5.07	5.12	5.14

[1]The values were calculated according to the values of feedstuffs (NRC 1994) except for crude protein and amino acids; CP was calculated from analyzed CP contents of

feedstuffs; digestible amino acids were calculated by digestible coefficients (Ajinomoto Heartland LLC, 2009) and analyzed amino acid contents of the ingredients plus the values of synthetic amino acids (digestible coefficients = 100%).

4.5 Results and discussion

4.5.1 Period I (from hatch to 21 d of age)

Standardized digestible amino acid values in the basal diet are shown in Table 4.7. The result indicated that the values of Lys, Met, Cys, Thr, Arg, Ile, Val, His, and Phe were similar to the calculated true digestible values from analyzed total amino acids with digestible coefficients reported by Ajinomoto Heartland LLC (2009) (Table 4.2). Only the value of Leu was slightly lower than the calculated value.

Table 4.7 Standardized digestible amino acid values of the diets provided to Korat chickens in period I, II, III, and IV. Values are expressed as % as fed.

Item	Period I (1-21 days of age)	Period II (22-42 days of age)	Period III and IV (43-63 and 64-84 days of age)
Lys	1.09	1.05	0.97
Met	0.32	0.29	0.26
Cys	0.31	0.30	0.26
Thr	0.79	0.69	0.68
Arg	1.21	1.26	1.21
Ile	0.81	0.76	0.74
Val	1.00	0.90	0.74
Leu	1.71	1.50	1.46
His	0.64	0.61	0.52
Phe	0.97	0.90	0.78

The current data show clearly that the Korat chickens responded significantly as dietary SAA levels increased in final BW (*P*<0.001), BW gain (*P*< 0.001), FCR (*P*=0.031), and UA in plasma (*P*<0.001), whereas no significant differences were observed in FI (*P*=0.124) between experimental treatments (Table 4.8). Protein deposition in the whole-body also significantly increased (*P*<0.001) as the SAA levels increased (Table 4.9). Likewise, increasing dietary SAA levels significantly improved (*P*≤0.047) the deposition of all selected amino acids such as Lys, Met+Cys, Thr, Arg, Ile, Val, Leu, His, and Phe (Table 4.9).

We observed a significant reduction in BW gain in birds fed a diet containing 0.63% digestible SAA compared to 0.73 0.83 0.93 and 1.03%. Clearly, it indicated this SAA level (0.63%) contains an insufficient amount to ensure protein accretion to further achieve an optimum BW gain of Korat chickens. The declined in chick growth associated with SAA-deficient diets has been shown to be mainly due to lower levels of whole-body protein synthesis with reduced RNA efficiency, which suggests translational regulation (Barnes et al., 1995). Indeed, the first step of the initiation of mRNA translation consists of the binding of initiator Met-tRNAi to the 40S ribosomal subunit to form the 43S preinitiation complex. This initiation step may be inhibited by methionine deficiency (Métayer et al., 2008). A significant improvement in FCR relative to the increase in dietary SAA concentrations is attributed to the significant increase in BW gain, but not FI. Dietary SAA deficiency caused to reduced BW gain and negative impact on FCR is consistent with previous studies (Knowles and Southern, 1998; Garcia and Batal, 2005; Lumpkins et al., 2007; Goulart et al., 2011; Dozier and Mercier, 2013).

The result of this period clearly shows that the UA reduced significantly as dietary SAA increased. Uric acid reached the highest level in the basal diet-fed birds (0.63% digestible SAA) and was significantly higher compared to UA of birds fed diets containing 0.73 0.83 0.93 and 1.03% digestible SAA. This can be explained that all experimental diets were formulated at the constant level of the essential amino acids except for Met. The efficacy of these amino acids is therefore limited because of a deficiency in dietary methionine. Consequently, experimental birds fed on a diet containing inadequate SAA level (0.63%) result in a higher UA than birds fed a diet containing adequate or higher SAA content. Clearly, UA in plasma decreased and BW gain increased as dietary SAA increased to the required level. However, no significant changes occurred as dietary SAA levels increased beyond the requirement. This finding was consistent with the reports by Miles and Featherston (1974) and Donsbough et al. (2010).

Table 4.8 Growth performance of Korat chickens from 1 to 21 days of age fed different sulfur amino acid levels. Unless otherwise indicated, values are expressed as g/bird.

Item	**Level of dietary sulfur amino acids**					**SEM**	***P*-value**
	0.63%	**0.73%**	**0.83%**	**0.93%**	**1.03%**		
Body weight							
Initial	41.83	41.84	41.78	41.75	41.82	0.038	0.942
Final	249.4^{b}	263.9^{a}	261.1^{a}	266.7^{a}	261.9^{a}	1.491	<0.001
Gain	207.5^{b}	222.1^{a}	219.3^{a}	224.9^{a}	220.1^{a}	1.498	<0.001
Feed intake	351.2	360.9	338.8	357.3	351.2	2.826	0.124
FCR, g/g	1.69^{a}	1.63^{ab}	1.54^{b}	1.59^{ab}	1.60^{ab}	0.015	0.031
Uric acid, mg%	7.63^{a}	5.92^{b}	5.72^{b}	5.27^{b}	5.18^{b}	0.213	<0.001

$^{a, b}$Means within each row with different superscripts are significantly different ($P \leq 0.05$).

BWG = body weight gain; FCR = feed converion ratio; SEM = standard error of the mean.

Likewise, the increase in dietary SAA from 0.73 to 1.03% showed no significant differences in PD and SAAD among treatments, whereas there were significant improvements in relation to the insufficient level (0.63%). In this respect, these results are in line with findings on BW gain and UA in plasma. This maybe due to the fact that SAA regulates protein turnover including protein synthesis and degradation, and therefore protein deposition (Métayer et al., 2008). The improvement in the deposition of protein/ amino acid of Korat chickens may also be due to increased IGF-I concentration, which plays a key role in protein synthesis and further in skeletal muscle development as well as the growth rate (Adams, 2002; Zanou and Gailly, 2013). It is clear that the addition of Met leads to an increase in the effectiveness of using other essential amino acids. As a result, increasing protein/ amino acid through the increased protein synthesis while inhibiting proteolysis in the whole-body of chicks.

The linear broken-line model was found to be the most appropriate to estimate the optimal SAA requirements for broilers (Millecam et al., 2021) and has been used in most studies of estimation of SAA requirements. Overall, some values of R^2 in the current study are quite low (i.e., from 0.15 to 0.67). However, this problem was also found in studies of Maliwan et al. (2019) and Millecam et al. (2021) and accepted for publication in Tropical Animal Health and Production and Poultry Science, respectively. In particular, R^2 values were from 0.26 to 0.75 (Maliwan et al., 2019) and from 0.10 to 0.84 (Millecam et al., 2021). The estimated optimal SAA levels in the periods of II, III, and IV were quite consistent among the different parameters, namely, from 0.74 to 0.76% in period II, from 0.66 to 0.68% in period III, and from 0.65 to 0.67% in period IV, whereas the period I were a range by 0.81 to 0.85%. The previous studies estimated amino acid requirements concluded the optimal amino acid according to three ways: the parameter of interest (Bernal et al., 2014), a combination of parameters (Dozier et al., 2010), or highest R^2 values (Millecam et al., 2021). In the case of this experiment, the author concluded the SAA requirement for Korat chickens based on the combination of parameters.

Table 4.9 Protein and anino acid deposition of Korat chickens from 1 to 21 days of age fed various digestible sulfur amino acid levels. Unless otherwise indicated, values are expressed as g/bird.

Item	**Level of digestible sulfur amino acids**					**SEM**	***P*-value**
	0.63%	**0.73%**	**0.83%**	**0.93%**	**1.03%**		
Protein	42.74^{b}	46.05^{a}	45.58^{a}	46.91^{a}	46.17^{a}	0.335	<0.001
Met+Cys	1.34^{b}	1.45^{ab}	1.51^{a}	1.52^{a}	1.52^{a}	0.018	0.001
Lys	2.30^{b}	2.46^{a}	2.51^{a}	2.60^{a}	2.51^{a}	0.029	0.004
Thr	1.41^{b}	1.51^{ab}	1.53^{ab}	1.63^{a}	1.56^{ab}	0.024	0.047
Arg	2.42^{b}	2.43^{b}	2.55^{ab}	2.67^{a}	2.48^{ab}	0.031	0.035
Ile	1.27^{b}	1.39^{ab}	1.41^{ab}	1.43^{ab}	1.48^{a}	0.022	0.013
Val	1.50^{b}	1.58^{ab}	1.61^{ab}	1.68^{a}	1.66^{a}	0.020	0.017
Leu	2.32^{b}	2.45^{ab}	2.50^{a}	2.57^{a}	2.53^{a}	0.025	0.005
His	1.56^{b}	1.69^{ab}	1.73^{a}	1.74^{a}	1.74^{a}	0.022	0.022
Phe	1.52^{b}	1.57^{ab}	1.66^{ab}	1.72^{a}	1.66^{ab}	0.023	0.018

[a, b] Means within each row with different superscripts are significantly different ($P \le 0.05$).

SEM = standard error of the mean.

The digestible SAA requirement of Korat chickens aged 1 to 21 days was estimated at 0.83 0.81 0.85 0.85 and 0.83% for BW gain, FCR, UA, PD, and SAAD, respectively (Table 4.10). Generally, the SAA requirements of Korat chickens from 1 to 21 days of age in the current study were very close to the requirements of Cobb 500 male broilers from 1 to 21 d of age (Garcia and Batal, 2005), i.e., between 0.83 and 0.88% and the digestible SAA requirements of Cobb 500 as recommended by the Cobb broiler management guide (2015), that is 0.88% (in the period 0-10 days of age) and 0.80% (in the period 11-22 days of age). Interestingly, the digestible SAA requirements of Korat chickens were similar to those for commercial broilers in NRC (1994). This is different from lysine requirements that indicated lysine requirements of Korat chickens were much higher than the need for commercial broiler in NRC (1994). However, the optimum SAA levels for Korat chickens in the present study were significantly higher than for fast-growing broilers in other reports. For example, Lumpkins et al. (2007) estimated the SAA requirements of Cobb 500 (both male and female) from 7 to 19 d of age at 0.67% and 0.71% for BW gain and gain to feed ratio, respectively. In addition, Goulart et al. (2011) observed male Cobb from 8 to 21 d of age required a dietary SAA level at 0.76%. Indeed, the digestible SAA requirements of Korat chickens are similar or higher than for commercial broilers is proven clearly when expressed SAA intake/BWG. In particular, SAA intake/BWG of Korat chickens (12.8 mg/g). This value was 11.0% higher than the value (i.e., 11.5 mg/g) of Cobb 500 male broilers aged 1-21 (Garcia and Batal, 2005), 54.5% higher than the value (i.e., 8.3 mg/g) of Cobb 500 aged 7-19 days Lumpkins et al. (2007), and 16.12% higher than the value (i.e., 11.1 mg/g) of Cobb aged 8-21 days (Goulart et al., 2011). Clearly, the nutrient requirements of Korat chickens are high as a result of their low feed consumption. As such, it must increase the concentration of nutrients in diets to satisfy the demand of Korat chickens. In addition, current data show that the SAA requirement for Korat chickens was similar or higher to that for fast-growing broilers in previous reports to be most likely due to the difference in the proportion of feathers between Korat chickens and fast-growing broilers. Indeed, the data in another experiment showed that the percentage of Cys in Korat chickens is 14.6% higher than Cobb 500 broilers. This statement was in line with the report by Kalinowski et al. (2003) that found Cys requirement for fast-feathering was higher than for slow-feathering, i.e., 12.8%. In contrast, when compared with the current guidelines, the optimum dietary SAA levels of Korat chickens from 1 to 21 days of age were lower than those of Ross, namely, 0.95% digestible SAA in the period 0-10 days of age and 0.87% in the period 11-24 days of age (Aviagen, 2019).

Table 4.10 The optimum levels of sulfur amino acids for Korat chickens from 1 to 21 days of age estimated by the linear broken-line model.

Item	**Equation**[1]	**Estimated requirement**	***P*-value**	**R^2**
BWG	$Y = 222.5 - 59.00 \times (0.8347 - x)$	0.83	0.0027	0.2394
FCR	$Y = 1.577 + 0.650 \times (0.8061 - x)$	0.81	0.0094	0.1498
UA	$Y = 5.225 + 9.583 \times (0.8549 - x)$	0.85	<0.0001	0.4689
PD	$Y = 46.54 - 14.21 \times (0.8533 - x)$	0.85	0.0002	0.3668
SAAD	$Y = 1.519 - 0.825 \times (0.8335 - x)$	0.83	<0.0001	0.5545

[1]The linear broken-line model was fitted as $Y = L + U \times (R - x)$, where Y is the dependent variable; x is the dietary sulfur amino acid level as independent variable; R is the optimum response of dietary sulfur amino acid; L is the response at x = r; and U = the steepness of the curve. In this model, Y = L when x > R.

BWG = body weight gain, FCR = feed conversion ratio, UA = uric acid, PD = protein deposition, SAAD = sulfur amino acid deposition.

4.5.2 Period II (22-42 days of age)

Standardized digestible amino acid values in the basal diet are shown in Table 4.7. The result indicated that the values of Lys, Cys, Arg, Ile, Val, His, and Phe were close to the calculated true digestible values from analyzed total amino acid with digestible coefficients reported by Ajinomoto Heartland LLC (2009) (Table 4.4), whereas the value of Met was slightly higher but Thr and Leu were lower than those calculated values in Table 4.4. Nevertheless, the value of Met+Cys was similar to the calculated value.

Korat chickens aged 22 to 42 days responded significantly to the final BW (P<0.001), BW gain (P<0.001), and FCR (P=0.001) as increasing dietary SAA levels from 0.58% to 0.90%, whereas FI was not significantly different (P=0.607) among experimental treatments (Table 4.11). The result clearly showed that the Korat chickens were fed with a low SAA diet (0.58%) was insufficient amounts for growth rate, but when dietary SAA levels in excess for requirement, its growth rate was not improved compared to the adequate level in the diet. In addition, the FCR of birds was improved significantly when fed on diets containing dietary SAA from moderate to high levels (0.74 0.82 and 0.90%) compared to low dietary SAA content (0.58%). Unlike period I, the UA in plasma was not significantly different (P=0.600) among experimental treatments (Table 4.11). Actually, not all research indicates that UA in plasma can be used as an indicator of the amino acid requirement of chickens. For example, Xie et al. (2004) reported both increases and decreases in concentrations of UA in plasmas when Met was increased in the diet. Miles and Featherston (1974) reported decreases in UA in plasma when dietary Lys was increased in the diet. Corzo et al. (2003, 2005) reported no changes in concentrations of UA in plasma when dietary Lys or Trp was increased in the diet.

Table 4.11 Growth performance of Korat chickens from 22 to 42 days of age fed different digestible sulfur amino acid levels. Unless otherwise indicated, values are expressed as g/bird.

Item	Level of dietary sulfur amino acids					SEM	*P*-value
	0.58%	**0.66%**	**0.74%**	**0.82%**	**0.90%**		
Body weight							
Initial	286.1	286.9	286.9	287.2	287.2	0.662	0.990
Final	661.9^{b}	671.7^{ab}	686.7^{a}	684.6^{a}	685.4^{a}	2.439	<0.001
Gain	375.7^{b}	384.8^{ab}	399.8^{a}	397.4^{a}	398.2^{a}	2.378	<0.001
Feed intake	917.3	907.4	904.4	889.5	888.9	6.442	0.607
FCR, g/g	2.44^{a}	2.36^{ab}	2.26^{b}	2.24^{b}	2.23^{b}	0.021	0.001
Uric acid, mg%	4.82	5.57	4.85	5.08	4.87	0.165	0.600

[a, b]Means within each row with different superscripts are significantly different ($P \leq 0.05$).

BWG = body weight gain; FCR = feed converion ratio; SEM = Standard error of the mean.

The PD in the whole-body was significantly affected ($P<0.001$) by an increase in dietary SAA (Table 4.12). In particular, the PD of birds fed a diet containing SAA at the moderate level (0.74%) increased significantly in comparison from a low SAA (0.58%). However, with the increase in dietary SAA levels from 0.82 to 0.90%, there was no further improvement in PD. Likewise, the deposition of amino acids differs significantly ($P<0.05$) among dietary SAA levels. These results were entirely consistent with the conclusion on BW gain.

Table 4.12 Protein and amino acid deposition of Korat chickens from 22 to 42 days of age fed various digestible sulfur amino acid levels. Unless otherwise indicated, values are expressed as g/bird.

Item	**Level of dietary sulfur amino acids**					**SEM**	***P*-value**
	0.58%	**0.66%**	**0.74%**	**0.82%**	**0.90%**		
Protein	75.99^{b}	81.02^{ab}	85.64^{a}	86.02^{a}	86.15^{a}	0.915	<0.001
Met+Cys	2.98^{b}	3.17^{ab}	3.39^{a}	3.43^{a}	3.36^{a}	0.049	0.002
Lys	4.08^{b}	4.32^{ab}	4.64^{a}	4.62^{a}	4.66^{a}	0.069	0.007
Thr	2.66^{b}	2.76^{ab}	2.97^{ab}	2.99^{ab}	3.03^{a}	0.046	0.016
Arg	4.21	4.32	4.72	4.70	4.65	0.073	0.047
Ile	2.55^{b}	2.86^{ab}	3.06^{a}	3.03^{ab}	3.09^{a}	0.066	0.027
Val	2.98^{b}	3.33^{ab}	3.44^{ab}	3.56^{a}	3.45^{ab}	0.069	0.047
Leu	3.82^{b}	4.14^{ab}	4.43^{a}	4.19^{ab}	4.35^{ab}	0.070	0.036
His	2.64^{b}	2.81^{ab}	3.10^{a}	3.04^{a}	3.08^{a}	0.052	0.003
Phe	2.86^{b}	2.96^{ab}	3.30^{a}	3.28^{a}	3.26^{a}	0.053	0.002

$^{a, b}$Means within each row with different superscripts are significantly different ($P \le 0.05$).

SEM = Standard error of the mean.

Using the broken-line regression analysis, the estimated digestible SAA requirements of Korat chickens from 22 to 42 days of age in the current study were 0.74 0.76 0.75 and 0.74 based on BW gain, FCR, PD, and SAAD, respectively. The result observed in this period was similar to the requirement of commercial broilers as in reports by Goulart et al. (2011), which indicated that the digestible SAA was required at 0.75% for male Cobb aged 22 to 35 days. In addition, in comparison with the current guidelines, the optimum SAA levels for Korat chickens from 22 to 42 days of age were very close to the digestible SAA requirements of Cobb 500 as recommended by the Cobb broiler management guide (2015), that is 0.74% (in the period 23-42 days of age). However, the SAA requirements for Korat chickens in this period were lower than those of Ross in the current guidelines, that is 0.80% digestible SAA in the period 25-39 days of age (Aviagen, 2019). In contrast, the digestible SAA requirement for Korat chickens in this period was higher than that in other studies. Particularly, NRC (1994) reccomended total SAA level for fast-growing broilers at 0.72%. Baker et al. (1996) estimated the digestible SAA requirements of Ross × Hubbard from 22 to 43 days of age were 0.63%. Furthermore, Lumpkins et al. (2007) indicated that SAA requirements of Cobb 500 from 21 to 42 days of age for males and females were 0.55 and 0.56%, respectively. In addition, the optimal digestible SAA level was found to be 0.66% in Ross308 male in the grower period (11-23 d) by Millecam et al. (2021). Indeed, the SAA requirements are expressed as SAA intake/BWG of Korat chickens in this period were so high, that is 16.7 mg/g in comparison with the values of 14.4 mg/g in NRC (1994), 10.4 mg/g in Baker et al. (1996), 8.4 mg/g in Lumpkins et al. (2007), and 12.5 mg/g in Goulart et al. (2011). Besides the reasons were presented above in period I, the SAA requirement for Korat chickens in this period was similar or higher to that for fast-growing broilers most likely because of environmental conditions, i.e., tropical climate. All birds in this period were raised in an open-sided, naturally ventilated barn and at around 33-38^{0}C. According to Dibner et al. (1992) indicated the total epithelial uptake of Met is decreased by 34% in the intestines of birds (from 26 to 42 days of age) raised in high environments (32^{0}C). In addition, Mitchell and Carlisle (1989) indicated amino acid absorption in the gut was reducted when chicks were kept in hot temperature because the size of the absorptive compartment was decreased by heat stress as reflected by decreased villus heights (19%) and wet (26%) and dry (31%) weight per unit length of jejunum. Therefore, the SAA requirement for Korat chickens was raised in hot environments might have been altered due to changes in gut morphology, amino acid absorption, or amino acid metabolism. Furthermore, the SAA requirements of Korat chickens in the current study were higher than commercial broilers being likely related to FI. It is well established that high ambient temperature caused a reduction in FI to maintain homeothermy (Han and Baker, 1993; Gonzalez-Esquerra and Leeson, 2006) and therefore required a higher SAA content in the diet. The evidence is FI of Korat chickens in this period reduced by 7.4% compared to FI Korat chickens were raised in the experiment of lysine requirement in the same period. Indeed, Silva et al. (2006) demonstrated that SAA requirement for Ross female broilers from 1 to 21 and 22 to 42 day of age raising at a high temperature requires higher SAA consumption to achieve optimal growth performance. However, it also needs to be noted that the temperature level that induces heat stress was different between Korat chickens and broilers. Therefore, future studies are required to determine which ambient temperature exceeds the tolerable range of Korat chickens as well as the relationship among heat stress, FI, and SAA requirements.

4.5.3 Period III (43-63 days of age)

Standardized digestible amino acid values in the basal diet estimated in Korat chickens from 43 to 63 and from 64 to 84 days of age are presented in Table 4.7. The data showed that the standardized digestible values of Cys, Leu, and His were closed to the calculated true digestible values derived from the total amino acid analyzed from feedstuffs (corn, soybean meal, and corn gluten meal) with digestible coefficients reported by Ajinomoto Heartland LLC (2009) (Table 4.6), whereas the values of Lys, Met, Arg, Ile, and Phe were higher but Thr and Val were lower than those calculated values in Table 4.6.

Table 4.13 The optimum levels of sulfur amino acids for Korat chickens from 22 to 42 days of age estimated by the linear broken-line model.

Item	**Equation**[1]	**Estimated requirement**	***P*-value**	**R^2**
BWG	Y = 397.9 – 143.4 × (0.740 – x)	0.74	<0.001	0.4044
FCR	Y = 2.235 + 1.146 × (0.764 – x)	0.76	<0.001	0.4585
UA	NE[2]			
PD	Y = 86.09 – 60.32 × (0.746 – x)	0.75	<0.0001	0.5284
SAAD	Y = 3.395 – 2.609 × (0.743 – x)	0.74	0.0002	0.4756

[1]The linear broken-line model (LBL) was fitted as $Y = L + U \times (R - x)$, where Y is the dependent variable; x is the dietary Lys level as independent variable; R is the optimum response of dietary Lys; L is the response at x = r; and U = the steepness of the curve. In this model, Y = L when x > R.

BWG = body weight gain, FCR = feed conversion ratio, UA = uric acid, PD = protein deposition, SAAD = Sulfur amino acid deposition.

[2]Not estimated because data did not conform to the regression model.

The Korat chickens in this period significantly responded on final BW (*P*<0.001), BW gain (*P*<0.001), FCR (*P*<0.001) as increasing SAA contents in the diets from 0.50% to 0.82%, whereas FI (*P*=0.200) and UA in plasma (*P*=0.324) were not significantly different among experimental treatments (Table 4.14). Body weight gain of Korat chickens increased significantly when fed diets containing SAA from 0.66 to 0.82% compared to 0.50% but BW gain remained relatively constant among treatments with 0.66 0.74 and 0.82% SAA. Similarly, FCR was significantly improved when Korat chickens were fed a diet containing 0.66% digestible SAA compared to 0.50% but no further improvement in FCR when dietary SAA content was increased to 0.82%. These results clearly demonstrated that the diet contains 0.50% digestible

SAA was inadequate for the growth rate of Korat chickens. Since the decrease in BW gain, whereas FI was constant, resulted in an increase in FCR when Korat chickens received insufficient SAA in the diet.

Table 4.14 Growth performance of Korat chickens from 43 to 63 days of age fed different digestible sulfur amino acid levels . Unless otherwise indicated, values are expressed as g/bird.

Item	Level of dietary sulfur amino acids					SEM	*P*-value
	0.50%	0.58%	0.66%	0.74%	0.82%		
Body weight							
Initial	678.8	679.4	679.8	679.9	678.8	1.351	0.998
Final	1079^{c}	1101^{bc}	1128^{ab}	1135^{a}	1121^{ab}	4.641	<0.001
Gain	400.5^{c}	421.6^{bc}	447.9^{ab}	455.1^{a}	442.4^{ab}	4.671	<0.001
Feed intake	1288	1277	1223	1287	1217	12.95	0.200
FCR, g/g	3.22^{a}	3.03^{ab}	2.73^{c}	2.83^{bc}	2.75^{c}	0.044	<0.001
Uric acid, mg%	5.27	5.67	4.85	4.42	5.35	0.222	0.324

$^{a, b, c}$ Means within each row with different superscripts are significantly different ($P \leq 0.05$).

BWG = body weight gain; FCR = feed converion ratio; SEM = Standard error of the mean

Likewise, the increase in dietary SAA levels significantly improved ($P<0.05$) the deposition of protein and selected amino acids such as Lys, Met+Cys, Thr, Arg, Ile, Val, Leu, His, and Phe (Table 4.15). The evidence clearly showed that a diet containing 0.50% digestible SAA did not contain sufficient content for synthesis protein. As a result, the deposition of protein and amino acid in the whole-body of Korat chickens decreased significantly compared to the diets containing from 0.66 to

0.82% SAA. The results of the protein and SAA deposition in the whole-body of Korat chickens are in line with the finding on BW gain.

Table 4.15 Protein and anino acid deposition of Korat chickens from 43 to 63 days of age fed various digestible sulfur amino acid levels. Unless otherwise indicated, values are expressed as g/bird.

Item	Level of dietary sulfur amino acids					SEM	*P*-value
	0.50%	**0.58%**	**0.66%**	**0.74%**	**0.82%**		
Protein	81.44^{a}	89.15^{ab}	94.61^{a}	95.46^{a}	96.81^{a}	1.307	<0.001
Met+Cys	3.34^{c}	3.65^{bc}	3.99^{ab}	4.04^{ab}	4.07^{a}	0.076	<0.001
Lys	4.28^{b}	4.88^{ab}	5.04^{a}	5.12^{a}	5.06^{a}	0.095	0.013
Thr	2.56^{b}	2.84^{ab}	3.07^{a}	3.11^{a}	3.08^{a}	0.061	0.004
Arg	4.37^{b}	4.77^{ab}	5.16^{ab}	5.26^{a}	5.27^{a}	0.109	0.014
Ile	2.60^{b}	3.09^{ab}	3.28^{ab}	3.32^{a}	3.34^{a}	0.090	0.028
Val	2.19^{b}	2.67^{ab}	2.86^{ab}	2.90^{a}	2.91^{a}	0.090	0.029
Leu	4.14^{b}	4.62^{ab}	4.84^{a}	4.85^{a}	4.90^{a}	0.086	0.009
His	3.11^{b}	3.63^{ab}	3.86^{ab}	3.96^{a}	3.81^{ab}	0.102	0.044
Phe	2.91^{b}	3.50^{ab}	3.66^{a}	3.80^{a}	3.57^{a}	0.091	0.007

$^{a, b, c}$ Means within each row with different superscripts are significantly different (P≤0.05).

FCR = feed converion ratio; SEM = Standard error of the mean

Using the broken-line regression analysis, the estimated digestible SAA requirements were 0.67 0.66 0.67 and 0.68% for BW gain, FCR, PD, and SAAD, respectively (Table 4.16). The optimum SAA levels for Korat chickens from 43 to 63 days of age were close to the digestible SAA requirements of Cobb 500 as recommended by the Cobb broiler management guide (2015), that is 0.70% (in the period from 43 days of age to market). Because fast-growing broilers usually had a finisher period at 35 or 42 days of age, there has no information to compare the SAA requirement between Korat chickens in this period and commercial broilers at the same age. However, Korat chickens aged 43 to 63 days can be considered finisher period. Therefore, the author discussed based on the same finisher period. The digestible SAA requirement of Korat chickens in this period was close to Cobb males in the final phase (from 36 to 42 days), which required 0.66% digestible SAA (Goulart et al., 2011). However, the SAA requirements for Korat chickens in this period were lower than those of Ross in the current guidelines, that is 0.75% digestible SAA in the period from 40 days of age to market (Aviagen, 2019). In contrast, the estimated SAA requirement of Korat chickens in this period was higher than that recommended by NRC (1994) for commercial broilers from 6 to 8 weeks of age, that is 0.60% total SAA. This statement is proven clearly when SAA requirements are expressed as SAA intake/BWG, i.e., 18.0 mg/g in the current study compared to 16.5 mg/g in NRC (1994).

Similar to the reasons that were presented in periods I and II, the SAA requirements for Korat chickens in this period were similar or higher than that for fast-growing boilers due to the difference in the proportion of feathers between Korat chickens and commercial broilers, and raising environmental conditions. Indeed, the FI of Korat chickens in this experiment reduced 13.3% in comparison with that in the experiment of lysine requirement because the temperature in this period was around 33-41^{0}C, whereas it was under 33^{0}C in the experiment of lysine requirement.

Table 4.16 The optimum levels of sulfur amino acids for Korat chickens from 42 to 63 days of age estimated by the linear broken-line model.

Item	**Equation**[1]	**Estimated requirement**	***P*-value**	$\mathbf{R^2}$
BWG	$Y = 448.8 - 296.0 \times (0.6659 - x)$	0.67	<0.0001	0.4348
FCR	$Y = 2.782 + 2.740 \times (0.6600 - x)$	0.66	<0.0001	0.4549
UA	NE[2]			
PD	$Y = 96.13 - 82.35 \times (0.6737 - x)$	0.67	<0.0001	0.5544
SAAD	$Y = 4.055 - 4.078 \times (0.6775 - x)$	0.68	<0.0001	0.6391

[1]The linear broken-line model (LBL) was fitted as $Y = L + U \times (R - x)$, where Y is the dependent variable; x is the dietary Lys level as independent variable; R is the optimum response of dietary Lys; L is the response at x = r; and U = the steepness of the curve. In this model, Y = L when x > R.

BWG = body weight gain, FCR = feed conversion ratio, UA = uric acid, PD = protein deposition, SAAD = Sulfur amino acid deposition.

[2]Not estimated because data did not conform to the regression model.

4.5.4 Period IV (64-84 days of age)

The response of Korat chickens in this period to dietary SAA on growth performance and plasma UA are shown in Table 4.17. In particular, the final BW, BW gain, FCR, and plasma uric acid were influenced by dietary treatments (P<0.05), whereas FI had no significant difference among treatments. As the dietary SAA increased from 0.50% to 0.66%, the BW gain increased significantly but there were no significant differences in BW gain of birds fed diets of 0.66%, 0.74%, and 0.82% SAA. In contrast, values on the FCR decreased significantly with increasing in dietary SAA contents. Particularly, FCR decreased significantly when birds fed on 0.66% SAA level compared to 0.50% in the diet. However, there have no significant differences in FCR of birds fed on the diet containing higher dietary SAA. Similarly, plasma UA in Korat chickens was significantly affected by dietary SAA. Birds were fed with the 0.58% SAA level had the lowest UA in plasma but it is no significant difference when dietary SAA content increase from 0.58% to 0.82%.

Table 4.17 Growth performance of Korat chickens from 64 to 84 days of age fed different digestible sulfur amino acid levels. Unless otherwise indicated, values are expressed as g/bird.

Item	Level of dietary sulfur amino acids					SEM	*P*-value
	0.50%	**0.58%**	**0.66%**	**0.74%**	**0.82%**		
Body weight							
Initial	1085.6	1086.0	1085.8	1086.3	1085.9	1.986	1.000
Final	1491^{b}	1516^{ab}	1541^{a}	1534^{a}	1530^{a}	4.750	0.003
Gain	406.0^{b}	429.5^{ab}	454.7^{a}	447.7^{a}	443.8^{a}	4.469	0.001
Feed intake	1470	1473	1445	1428	1435	10.69	0.597
FCR	3.63^{a}	3.43^{ab}	3.18^{b}	3.19^{b}	3.24^{b}	0.046	0.001
Uric acid, mg%	5.52^{a}	3.57^{b}	4.00^{ab}	4.22^{ab}	4.93^{ab}	0.209	0.014

$^{a, b}$ Means within each row with different superscripts are significantly different ($P \le 0.05$).

BWG = body weight gain; FCR = feed converion ratio; SEM = Standard error of the mean.

The response of Korat chickens from 64 to 84 days of age to dietary SAA on the deposition of protein and selected amino acids in the whole-body is shown in Table 4.18. There was a significant increase in birds fed with 0.58% SAA compared to 0.50% but there were no significant differences when the dietary SAA increased from 0.58% to 0.82%. The deposition of Met+Cys, Lys, Thr, Arg, Ile, Val, Leu, His, and Phe increased significantly when birds fed with 0.66% SAA compared to 0.50% but there had no significant difference when dietary SAA increase from 0.66% to 0.82%.

Table 4.18 Protein and anino acid deposition of Korat chickens from 64 to 84 days of age fed various digestible sulfur amino acid levels. Unless otherwise indicated, values are expressed as g/bird.

Item	**Level of dietary sulfur amino acids**					**SEM**	***P*-value**
	0.50%	**0.58%**	**0.66%**	**0.74%**	**0.82%**		
Protein	81.92[b]	88.54[a]	98.45[a]	98.61[a]	97.65[a]	1.822	0.003
Met+Cys	3.51[c]	3.82[bc]	4.17[ab]	4.16[ab]	4.24[a]	0.071	<0.001
Lys	4.20[b]	4.74[ab]	5.11[a]	5.16[a]	5.12[a]	0.106	0.003
Thr	2.88[b]	3.15[ab]	3.43[a]	3.32[a]	3.29[ab]	0.054	0.002
Arg	4.50[b]	5.11[ab]	5.62[a]	5.63[a]	5.79[a]	0.129	0.001
Ile	2.53[b]	2.81[ab]	3.34[a]	3.32[a]	3.29[a]	0.100	0.010
Val	2.43[b]	2.74[ab]	3.16[a]	3.24[a]	3.15[a]	0.091	0.004
Leu	4.12[b]	4.46[ab]	5.10[a]	5.20[a]	5.05[a]	0.122	0.003
His	3.16[b]	3.71[ab]	4.09[a]	4.02[a]	4.05[a]	0.106	0.009
Phe	3.04[b]	3.35[ab]	3.88[a]	3.66[a]	3.60[a]	0.083	0.003

[a, b, c]Means within each row with different superscripts are significantly different ($P \leq 0.05$).
FCR = feed converion ratio; UA = uric acid; SEM = Standard error of the mean.

The estimated digestible SAA requirements for Korat chickens during 64-84 d period were 0.65 0.66 0.66 and 0.67%, respectively, for optimum BW gain, FCR, PD, and SAAD based on broken-line regression analysis (Table 4.19).

Unfortunately, the UA in plasma cannot be estimated because the data did not conform to the regression model. Generally, the SAA requirement of Korat chickens was higher than commercial broilers. In particular, Millecam et al. (2021) reported that male Ross 308 in the finisher phase (24-35) required 0.62% digestible SAA. The reasons why the estimated SAA requirement for Korat chickens was higher than that for Ross or Cobb were presented in detail in the previous periods. Clearly, because Korat chickens were raised in this experiment at high environmental temperatures (36-41^{0}C), it leads to the drop in FI 15.9% compared to FI of Korat chickens was raised in the experiment of lysine requirement with a moderate temperature (19-27^{0}C). This was proven by reports of Howlider and Rose (1987), which indicated the sharp decrease in FI at a rate of approximately 1.5% per 1ºC above 20ºC. Birds may try to limit heat production by decreasing FI to maintain homeothermy under heat stress. However, the mechanisms by which FI decreases at high temperatures are largely unknown. Yahav et al. (1996) reported that levels of triiodothyronine (T3) in plasma are highly correlated to FI and ambient temperature in birds. Plasma T3 levels fall immediately after heat exposure in birds presumably in order to reduce heat production and maintain homeothermy (Uni et al.,2001). These findings suggest that the dropped FI observed in heat stressed birds may also result from thermally-induced changes in plasma T3 levels, although conversely changes in plasma T3 could be a secondary response to a decreased FI.

As regard the SAA to Lys ratio, 77 77 74 and 78% were determined in periods I, II, III, and IV, respectively in the current study. These values were within the recommended range of previous studies. Which indicated 69% for Cobb 500 or Ross 308 aged 14 to 35 days (Vieira et al., 2004); 70% for Cobb aged 8 to 21 days (Goulart et al., 2011); 71% for Cobb aged 1 to 7 days (Goulart et al., 2011); 72% for

New Hampshire male x Columbian female aged 8 to 22 days (Baker and Han, 1994) or Cobb aged 36 to 42 days (Goulart et al., 2011); 74% for Ross × Ross 708 aged 1 to 15 days (Dozier and Mercier, 2013); 75% for ISA 220 or Ross 208 aged 20 to 40 days (Mack et al., 1999); 76% for Cobb aged 22 to 35 days; 77% for Hubbard × Cobb 500 aged 1 to 15 days (Dozier and Mercier, 2013); 82, 0.72, and 71% for Broilers aged 0-3, 3-6, and 6-8 wk, respectively (NRC, 1994). Overall, the SAA to Lys ratio in the current study was quite high. This could result from the high SAA requirement.

Table 4.19 The optimum levels of sulfur amino acids for Korat chickens from 64 to 84 days of age estimated by the linear broken-line model.

Item	**Equation**[1]	**Estimated requirement**	***P*-value**	**R^2**
BWG	$Y = 448.7 - 292.7 \times (0.6458 - x)$	0.65	0.0001	0.3031
FCR	$Y = 3.209 + 2.642 \times (0.6600 - x)$	0.66	0.0001	0.3436
UA	NE[2]			
PD	$Y = 98.13 - 103.4 \times (0.6622 - x)$	0.66	0.0002	0.3585
SAAD	$Y = 4.199 - 4.094 \times (0.6695 - x)$	0.67	<.00001	0.6719

[1]The linear broken-line model (LBL) was fitted as $Y = L + U \times (R - x)$, where Y is the dependent variable; x is the dietary Lys level as independent variable; R is the optimum response of dietary Lys; L is the response at x = r; and U = the steepness of the curve. In this model, Y = L when x > R.

BWG = body weight gain, FCR = feed conversion ratio, UA = uric acid, PD = protein deposition, SAAD = Sulfur amino acid deposition.

[2]Not estimated because data did not conform to the regression model.

When the SAA requirement expressed as a percent of the protein (%SAA/ %CP x 100). The SAA requirements for Korat chickens in the present experiments were found to be 3.9% (= 0.83%/ 21.26% x 100) in period I and 3.7% in periods II, III, and IV). The SAA requirement decreased from 3.9% during 1–21 days to 3.7% during 22-42 days of age agree with reports of NRC (1994) and Boomgaardt and Baker (1973), which requirement for SAA decreased in older chicks compared to younger chicks, i,e 3.9% (0.90% SAA/ 23% CP x 100 = 3.9%) during period 0-3 wk and 3.6% (0.72% SAA/ 20% CP x 100 = 3.6% during period 3-6 wk of age (NRC, 1994), and from 3.05% during 14-28 days to 2.56% during 42-56 days of age (Boomgaardt and Baker,1973). In contrast, the SAA requirements for Korat chickens were similar among periods II, III, and IV being consistent with the finding of Bornstein and Lipstein (1966), which reported the SAA requirement of 3.6% for broilers from 0 to 10 weeks of age. The values were observed in the current study to be similar to the recommendation by Boomgaardt and Baker (1973b) that the SAA need remained essentially constant at 4% of the CP.

Overall, dietary SAA was required for Korat chickens in the present study decreases with increasing age, i.e., 0.83 (from 1 to 21 days of age), 0.75 (from 22 to 42 days of age), 0.67 (from 43 to 63 days of age), and 0.66% (from 64 to 84 days of age). These observations were consistent with the reports of Boomgaardtd and Baker (1973a), NRC (1994), Lumpkins et al. (2007), Goulart et al. (2011), and Millecam et al. (2021). Differences in SAA requirements over the growing period were based upon differences in percentage growth rate and tissue maintenance requirements (Mitchell, 1959). In particular, BW of Korat chickens increases by 6.2 2.4 1.7 and 1.4-times at the end compared to the beginning of each period I, II, III, and IV, respectively.

According to the hypothesis (in chapter I), the optimum dietary sulfur amino acids levels for Korat chickens may differ among measured criteria. However, the results in the present study indicated that the SAA requirements for Korat chickens were similar among measured criteria (i.e., optimum levels for FCR, UA, PD, or SAAD were ≤2.4 lower or ≤3.1 higher in comparison with BW gain in all periods). In the previous studies that showed the SAA requirement is higher for obtaining optimum FCR than for maximum BW gain because both BW gain and FI increased until the point of optimum SAA levels for BW gain, then BW gain is a plateau, whereas FI still reduced when higher dietary SAA level than the point of optimum SAA levels for BW gain. Nevertheless, the FI of Korat chickens was not the same trend as that in those studies. This maybe the main reason why the SAA requirement for Korat chickens was not different between BW gain and FCR. Furthermore, it has been established that amino acid intake follows anabolic routes to tissue proteins, hormones, neurotransmitters, and other bioactive molecules or catabolic pathways to the amino group and carbon skeleton. The amino group creates uric acid, whereas the carbon skeleton of some amino acids (Lys, Thr, Leu, Phe, Trp) synthesizes fatty acid through Acetyl-CoA (Akers and Denbow, 2008). It maybe the number of synthesized fatty acid from the carbon skeleton of Lys, Thr, Leu, Phe, Trp is not much to create a significant difference between SAAD and growth performance. This finding in the current study was line in with the recent report of Goulart et al. (2011) and Millecam et al. (2021), which found that the optimal SAA level was similar between BW gain, FCR, and breast weight. However, the observations in our study differed from many previous reports that indicated the SAA requirement for fast-growing broilers is higher for obtaining optimum FCR or breast meat yield than for maximum BW gain

(Adams et al, 1962; Bornstein and Lipstein, 1964, 1966; Van Weerden et al, 1976; Schutte and Pack, 1995; Baker et al., 1996; Lumpkins et al., 2007).

4.6 Conclusions

Based on the present experiment, it can be concluded that the digestible SAA requirements for Korat chickens were 0.83, 0.75, 0.67, and 0.66% or 141, 335, 404, and 477 mg/bird/d in periods I (from 1 to 21 days of age), II (from 22 to 42 days of age), III (from 43 to 63 days of age), and IV (from 64 to 84 days of age), respectively, based on averages of the measured criteria. The optimal SAA levels for Korat appear to be similar among the measured criteria such as BW gain, FCR, UA, PD, and SAAD.

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CHAPTER V

OVERALL CONCLUSION AND IMPLICATION

5.1 Conclusion

The digestible lysine and SAA requirements for Korat chickens during 1-21 d (period I), 22-42 d (period II), 43-63 d (period III), and 64-84 d (period IV) based on growth performance, UA in plasma, protein deposition, and lysine and SAA depositions in the whole-body can be concluded as follows.

1) The digestible lysine requirements for Korat chickens were 108 1.03 1.12 1.09 and 1.10% for BW gain, FCR, UA in plasma, PD, and LysD, respectively, in period I; 0.97 1.00 0.95 0.96 and 1.00% for BW gain, FCR, UA, PD, and LysD, respectively, in period II; 0.93 0.93 0.89 and 0.90% for BW gain, FCR, PD, and LysD, respectively, in period III; and 0.82 0.87 0.88 0.84 and 0.85% for BW gain, FCR, UA in plasma, PD, and LysD, respectively, in period IV.

2) In addition to lysine requirement, it can be concluded that the SAA requirements for Korat chickens were 0.83 0.81 0.85 0.85 and 0.82% for BW gain, FCR, UA, PD, and SAAD, respectively, in period I; 0.74 0.76 0.75 and 0.74% for BW gain, FCR, PD, and SAAD, respectively, in period II; 0.67 0.66 0.67 and 0.68% for BW gain, FCR, PD, and SAAD, respectively, in period III; and 0.65 0.66 0.66 and 0.67% for BW gain, FCR, PD, and SAAD, respectively, in period IV.

3) Overall, the estimated lysine/ SAA requirements for Korat chickens were similar among parameters such as BW, PD, and LysD/ SAAD. The measurement

parameters of FCR and UA were also closed to BW gain except for Korat chickens aged 64-84 days in lysine requirement experiment. The author therefore recommended the digestible lysine requirements for Korat chickens should be 1.08 0.98 0.91 and 0.85% aged 1-21, 22-42, 43-63 and 64-84 days, respectively, on averages of the measured criteria. The digestible SAA requirements for Korat chickens are recommended at 0.83 0.75 0.67 and 0.66% based on the combination of measured criteria. However, feed producers may decide to use the dietary lysine/ SAA level depending on the parameter of interest.

5.2 Implication

From a scientific point of view, the determination of digestible lysine requirements for Korat chickens could provide valuable information for the establishment of an ideal amino acid profile for Korat chickens. From an environmental point of view, the exact level of inclusion of these amino acids could decrease a negative impact on the environment as a result of reduced nitrogen excretion. From an economic point of view, estimating the precise digestible lysine and SAA requirements for Korat chickens could increase profits through optimum growth performance and lower feed costs. In addition, feed manufacturers can use feedstuffs that contain low levels of lysine or methionine content as by-products (Corn DDGS, coconut meal, cassava pulp, etc…) from the industry or agriculture to formulate diets for Korat chickens. This makes it possible not only to reduce feed costs but also to solve food competition between humans and animals. From a scientific, environmental, and economic point of view, farmers, feed manufacturers, and nutritionists can apply this database to formulate the diets for Korat chickens, native chickens, and other indigenous crossbred chickens with growth rates similar to Korat chickens.

The present study estimated lysine and SAA requirements based on parameters of BW gain, FCR, UA in plasma, PD, and LysD/ SAAD, not include breast meat or thigh yield because in practical “Korat” chicken, currently marketed as a whole-body. Therefore, in the future, the lysine and SAA requirements for Korat chickens should be estimated according to the parameters of breast meat or thigh yield at 63 and 84 days of age when customers have a demand to consume parts of meat.

BIOGRAPHY

Mrs. Tran Hong Dinh was born on 9th February 1983 in Bac Lieu, Vietnam. In 2008, she graduated a Bachelor degree in Veterinary Medicine at Can Tho University (CTU), Vietnam. She was employed by the Department of Animal Science and Veterinary Medicine, College of Agriculture, Bac Lieu University. At the same time, she studied M.Sc program at CTU, Vietnam in the field of Animal Nutrition and graduated in 2010. From 2011 she has been a lecturer of Department of Animal Science and Veterinary Medicine, College of Agriculture, Bac Lieu University. In 2015, she got an award (Ph.D. program) from Mekong 1000 scholarship program by Vietnam government. She studied in the field of poultry nutrition at School of Animal Technology and Innovation, Institute of Agricultural Technology, Suranaree University of Technology, Thailand from November 2015 to October 2021 with the thesis entitled "Digestible lysine and sulfur amino acid requirements of Korat chickens". During her Ph.D. study, she has published one article "Tran, D. H., Schonewille, J. Th., Pukkung, C., and Khempaka, S. (2021). Growth performance and accretion of selected amino acids in response to three levels of dietary lysine fed to fast- and slow-growing broilers. Poult. Sci. 100(4): 100998."