STUDY ON THE REGULATORY MECHANISM INVOLVED IN THE MUSCLE DEVELOPMENT AND MEAT QUALITY BY IN OVO FEEDING OF L-ARGININE IN KORAT CHICKENS

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การศึกษากลไกที่เกี่ยวข้องในกระบวนการพัฒนาของกล้ามเนื้อและคุณภาพเนื้อด้วยวิธีการฉีด L-arginine ผ่านเปลือกไข่ในไก่โคราช

นางสาวพานพาน ลู

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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พานพาน ลู : การศึกษากลไกที่เกี่ยวข้องในกระบวนการพัฒนาของกล้ามเนื้อและคุณภาพเนื้อด้วยวิธีการฉีด L-arginine ผ่านเปลือกไข่ในไก่โคราช (STUDY ON THE REGULATORY MECHANISM INVOLVED IN THE MUSCLE DEVELOPMENT AND MEAT QUALITY BY IN OVO FEEDING OF L-ARGININE IN KORAT CHICKENS) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร. วิทธวัช โมพี, 103 หน้า.

การศึกษาในครั้งนี้ มีวัตถุประสงค์เพื่อศึกษาผลของการฉีด L-Arginine (Arg) ผ่านเปลือกไข่ต่อกระบวนการพัฒนาของกล้ามเนื้อ และคุณภาพเนื้อในไก่โคราช โดยการนำไข่ของไก่โคราชที่มีเชื้อจำนวน 480 ฟอง จัดแบ่งเป็น 2 กลุ่มการทดลอง คือ กลุ่มที่ 1 ไข่ที่ไม่ได้รับการฉีดสาร (กลุ่มควบคุม) และกลุ่มที่ 2 ไข่ที่ได้รับการฉีด Arg ผ่านเปลือกไข่ โดยการฉีดสารละลาย 0.5 มิลลิลิตรต่อฟอง (Agr 10 กรัม ในน้ำเกลือเข้มข้น 0.9% 1 ลิตร) ในวันที่ 18 ของระยะฟัก หลังจากการฟักออกสุ่มลูกไก่โคราชคละเพศที่มีสุขภาพดี กลุ่มการทดลองละ 160 ตัว แบ่งเป็น 4 ซ้ำ ๆ ละ 40 ตัว และเลี้ยงไก่โคราชจนสิ้นสุดการทดลองที่อายุ 63 วัน ผลการทดลองพบว่า การฉีด Arg ผ่านเปลือกไข่มีผลต่อส่วนประกอบของกรดอะมิโนในกล้ามเนื้ออกของไก่โคราช ($P<0.05$) ในช่วงอายุแรกเกิดจนถึงอายุ 42 วันหลังการฟัก ในส่วนการทำงานของเอนไซม์ที่เกี่ยวข้องกับการยับยั้งการเกิดอนุมูลอิสระ พบว่าการฉีด Arg ผ่านเปลือกไข่มีผลต่อการทำงานของเอนไซม์ในช่วงอายุแรกเกิดจนถึงอายุ 21 วัน ($P<0.05$) ทั้งเพิ่มการแสดงออกของยีน Myf5 MyoG และ MRF4 ที่เกี่ยวข้องกับการพัฒนาของกล้ามเนื้ออกในช่วงอายุแรกเกิดจนถึงอายุ 21 วัน อย่างไรก็ตามการฉีด Arg ผ่านเปลือกไข่ไม่มีผลต่อการฟักออก การเจริญเติบโต องค์ประกอบซาก สัณฐานวิทยาของกล้ามเนื้อ และการแสดงออกของยีน mTOR S6K1 4EBP1 Pax7 และ MyoD ตั้งแต่แรกเกิดจนถึงอายุ 63 วัน นอกจากนี้เมื่อนำการเจริญเติบโตของกล้ามเนื้อและยีนที่เกี่ยวข้องกับกระบวนการพัฒนาของกล้ามเนื้อมาวิเคราะห์ความสัมพันธ์ด้วยการวิเคราะห์องค์ประกอบหลัก (Principal Component Analysis, PCA) พบว่ามีความแตกต่างกันอย่างชัดเจนระหว่างกลุ่มควบคุมและกลุ่มที่ได้รับการฉีด Arg ผ่านเปลือกไข่ในช่วงอายุแรกเกิดและอายุ 21 วันหลังการฟักของไก่โคราช และพบว่ามีแนวโน้มที่จะเกิดความแตกต่างเช่นเดียวกันในช่วงอายุ 42 วัน และ 63 วันหลังการฟัก

จากการทดลองครั้งนี้สรุปได้ว่า การฉีด Arg ผ่านเปลือกไข่ไม่มีผลต่อการเจริญเติบโต องค์ประกอบซาก สัณฐานวิทยาของกล้ามเนื้อ และคุณภาพเนื้อ อย่างไรก็ตามสามารถปรับปรุงในส่วนของกล้ามเนื้ออกของไก่โคราชในช่วงแรกเกิด โดยมีผลต่อการทำงานของเอนไซม์ส่วนประกอบ กรดอะมิโน และการแสดงออกของยีนที่เกี่ยวข้องกับกระบวนการพัฒนาของกล้ามเนื้อ ดังนั้นการฉีด Arg ผ่านเปลือกไข่อาจนำไปสู่การสร้างความแตกต่างของกล้ามเนื้อ โดยการพัฒนาของเซลล์กล้ามเนื้อในรูปของ myotube ซึ่งเป็นผลดีต่อการพัฒนากล้ามเนื้อของไก่โคราช

อีกทั้งเทคนิคการฉีดสารผ่านเปลือกไข่ไม่มีผลเสียต่อคุณภาพเนื้อของไก่โคราช วิธีการนี้อาจถือได้ว่าเป็นวิธีที่ปลอดภัยและสามารถนำไปใช้ในการผลิตสัตว์ปีก

สาขาวิชาเทคโนโลยีและนวัตกรรมทางสัตว์ ลายมือชื่อนักศึกษา Pampan Lu
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PAN PAN LU: STUDY ON THE REGULATORY MECHANISM INVOLVED IN THE MUSCLE DEVELOPMENT AND MEAT QUALITY BY IN OVO FEEDING OF L-ARGININE IN KORAT CHICKENS. THESIS ADVISOR : ASST. PROF. WITTAWAT MOLEE, Ph.D., 103 PP.

ARGININE/IN OVO FEEDING/MUSCLE DEVELOPMENT/MEAT QUALITY/CHICKENS

This study was conducted to evaluate the effect of in ovo feeding (IOF) of L-Arginine (Arg) on muscle development and meat quality in Korat chickens. A total of 480 viable eggs were randomly divided to 2 treatment groups: 1) non-injected control group and 2) 1% Arg group, injected with 0.5 ml Arg solution (10 g Arg was dissolved in 1 L saline of 0.9%) on 18 days of incubation. After hatching, 160 mixed-sex and healthy birds of each treatment were randomly divided into 4 replicates of 40 birds each. This experiment lasted for 63 days. The results of this study showed the compositions of amino acid in breast muscle were affected ($P<0.05$) from DOH to D42 post-hatch. The antioxidant capacity was affected ($P<0.05$) during DOH to D21 post-hatch. The mRNA abundances of Myogenic factor 5 (Myf5), myogenin (MyoG) and muscle regulator 4 (MRF4) genes related to the muscle development in breast muscle were increased by IOF of Arg during DOH to D21 post-hatch. However, IOF of Arg did not affect the hatchability, growth performance, carcass traits, muscle fiber traits and mRNA expressions of mammalian target of rapamycin (mTOR) and their downstream genes of ribosomal protein S6 kinase (S6K1), eukaryotic initiation factor 4E binding protein 1 (4EBP1), paired box 7 (Pax7) and myogenic differentiation factor 1 (MyoD) post-hatch. In addition, muscle growth traits and genes of muscle

development were analyzed by principal component analysis (PCA) and showed that there is clear separation between control and Arg groups on DOH and D21, respectively, while a trend of grouping from two groups was exhibited on D42 and D63, respectively.

In conclusion, the results of this study indicated that IOF of Arg did not affect the growth performance, carcass traits, muscle fiber traits and meat quality. However, the antioxidant capacity, amino acid compositions, genes expression related to the muscle development were improved in breast muscle during starter period. Therefore, IOF of Arg may contribute to the muscle differentiation to form myotube rather than proliferation, which may had a positive role in muscle development of Korat chickens. In ovo technique had no negative effect on meat quality in Korat chickens and might be considered a safe way to apply in poultry production.

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LIST OF ABBREVIATIONS

Arg	=	L-arginine
Asp	=	Aspartic acid
BW	=	Body weight
BWG	=	Body weight gain
DOH	=	Day of hatch
4EBP1	=	eIF4E-binding protein 1
FCR	=	Feed conversion ratio
FI	=	Feed intake
GAPDH	=	Glyceraldehyde 3-phosphate dehydrogenase
Glu	=	Glutamic acid
Gly	=	Glycine
GSH	=	Glutathione
His	=	Histidine
Ile	=	Isoleucine
IOF	=	In ovo feeding
Leu	=	Leucine
Lys	=	Lysine

LIST OF ABBREVIATIONS (Continued)

MDA	=	Malondialdehyde
Met	=	Methionine
MFD	=	Muscle fiber diameter
MFN	=	Muscle fiber number
MRF4	=	Myogenic regulatory factor 4
mTOR	=	Mechanistic target of rapamycin
Myf5	=	Myogenic factor 5
MyoD	=	Myogenic differentiation factor
MyoG	=	Myogenin
NCBI	=	National Center for Biotechnology Information
Pax3	=	Paired box 3
Pax7	=	Paired box 7
Phe	=	Phenylalanine
Pro	=	Proline
Real-time PCR	=	Real-time polymerase chain reaction
SEM	=	Standard error of mean
Ser	=	Serine
S6K1	=	Ribosomal protein S6 kinase 1

LIST OF ABBREVIATIONS (Continued)

T-AOC	=	Total antioxidant capacity
Thr	=	Threonine
Tyr	=	Tyrosine
Val	=	Valine

CHAPTER I

INTRODUCTION

1.1 Introduction

With the improvement of people's living standard and increase of world population, consumption of meat production is increasingly year by year, it is supposed that population will continue to increase as well as food demand in the future (Godfray et al., 2010).

Chicken meat are favored by consumers that contribute to the higher protein and low fat. Generally, commercial chicken is fast-growing broiler strain that their body weight can get 2.5 kg on 6 weeks of age (Fanatico et al., 2005). This reduction of growing time and intensive muscle growth tend to cause the muscle damage (Barbut et al., 2008). Mainly because of the increase of muscle fiber size may be related to the lower capillarization, that cannot meet the maximum oxygen supply and nutrients to muscle cells, which furtherly induced high incidence of spontaneous, idiopathic myopathies or increased susceptibility to stress-induced myopathy (Petracci and Cavani, 2012), such as deep pectoral myopathy (DPM) and pale-soft-and-exudative (PSE)-like meat (Dransfield and Sosnicki, 1999; Duclos et al., 2007), leading to the decrease of meat quality and increase of economic loss.

Alternatively, slow-growing chickens are paid more attention by consumers and producers (Yang and Jiang, 2005). It is deemed that slow-growing chickens, are given more time to reach the market weight that may reduce the muscle abnormalities

(Devatkal et al., 2019), and can produce high-quality meat than fast-growing chickens (Sarsenbek et al., 2013). “Korat chicken” a slow-growing chicken, is Thai indigenous crossbreed strain that comes from Leung Hang Khao male chicken (Thai native chicken) and Suranaree University of Technology (SUT) female chicken. The body weight of Korat chickens reached 1.2 kg on 63 days of age. Their meat contains less fat, higher quantities of protein and have a good taste (Sangsawad et al., 2016; Hang et al., 2018). However, Korat chickens cannot be segmented by cutting through every parts to sell in the market, such as, breast muscle meat has less yield. So, increasing the meat yield with high meat quality are attracting our attention.

Skeletal muscle, is considered as the most abundant tissue in animals. Skeletal muscle development includes hyperplasia and hypertrophy. Hyperplasia is the increase of muscle fiber number during hatching (Smith, 1963). After hatching, muscle development is depend on the hypertrophy, which is the increase of muscle fiber size by the protein deposition that is derived from accumulation of satellite cells (Merly et al., 1998; Velleman, 2007). Both of them decide the muscle meat yield of chickens.

Muscle development could be affected by nutrition regiment in animals (Wen et al., 2014; Chen et al., 2018). Early nutritional program, as a strategy could provide excellent opportunity to improve the muscle development and keep this advantage to the marketing age. It has been proven that in ovo feeding (IOF) of appropriate nutrients (carbohydrates, amino acids, vitamins) enhanced embryonic and post-hatch performance (Zhai et al., 2011; Zhang et al., 2018; Araujo et al., 2019), play a positive role in muscle development and subsequent performance post-hatch (Kadam et al., 2013).

L-arginine (Arg) is essential amino acid for poultry, because of Arg cannot be synthesized by endogenous pathway. Arg participates in protein synthesis, and also is

precursor of some metabolic molecules, such as, nitric oxide (NO), creatine and proline, so Arg serves many biological and physiological functions (Khajali and Wideman, 2010). Previous studies indicated that dietary Arg positively affected muscle growth in starter phase (Fernandes et al., 2009). IOF of Arg improved the hatchability and growth performance (Gao et al., 2017; Zhang et al., 2017). Some studies have proved that the effects of IOF of Arg on breast muscle weight, genes expression of mTOR signaling pathway and myogenesis in broiler chickens in starter period (Li et al., 2016; Yu et al., 2018). Young animals have a particularly high requirement of Arg for growth and metabolic function (Flynn et al., 2002).

In order to increase the meat yield with maintaining the high quality of Korat chickens. Therefore, the objectives of this study were to study on the regulatory mechanism involved in the muscle development and meat quality by IOF of Arg in Korat chickens.

1.2 Research objectives

1.2.1 To study the effects of Arg on muscle development by IOF technique in Korat chickens.

1.2.2 To study the effects of Arg on meat quality by IOF technique in Korat chickens.

1.3 Research hypothesis

1.3.1 IOF of Arg can promote muscle development in Korat chickens.

1.3.2 IOF of Arg can positively affect the meat quality in Korat chickens.

1.4 Scope and limitations of the study

1.4.1 Arg will be used in this experiment.

1.4.2 Korat chickens from Leung Hang Khao male chicken and SUT female chickens will be used in experiments.

1.4.3 Feeding environment cannot be controlled, due to the chickens are raised in semi-open house, sometimes the temperature cannot be kept constant.

1.5 Expected benefits

1.5.1 The results of this study will improve the muscle mass of Korat chickens.

1.5.2 The results of this study could increase the meat quality, at least has no negative effect on meat quality of Korat chickens.

1.5.3 The technology of IOF of Arg may be applied on production of Korat chickens.

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CHAPTER II

LITERATURE REVIEWS

2.1 The structure of skeletal muscle

The muscle system includes four parts of skeletal muscle, connective tissue, nerve tissue and blood (vascular tissue). Skeletal muscle is considered as complex and heterogeneous tissue, performing multitude function in the organism (Bentzinger et al., 2012). The skeletal muscle formation contains the maintenance of stem and progenitor cell, lineage specification and terminal differentiation during the embryonic stage, (Bentzinger et al., 2012), and this process is finished when the animal grow up until adult stage. The skeletal muscle is composed by lots of bundled muscle fibers that come from myofibrils, the thick and thin filaments are contained in the myofibrils, which form into a contractile unit, called sarcomere, is surrounded by basal lamina and satellite cells are underneath it (Allen, 1978). Which are the stem cells of adult skeletal muscle that help to the muscle growth, repair and regeneration (Hikida, 2011; Macaluso and Myburgh, 2012). Mature muscle fibers are surrounded by endomysium, bundles of muscle fiber are wrapped by perimysium, many bundles are covered by epimysium, is connected to the bone via myotendinous junctions (Figure 2.1) (Shahjahan, 2015). Muscle includes well-defined muscle fibers and distinct spacing between muscle fibers bundles, these spacing plays an important role for preventing muscle fiber degeneration. In embryonic stage, muscle growth involves the increase of myofiber number. In adult stage, skeletal muscle are composed by

bundles of multinucleated myofibers, and that distribute tendon to tendon then perform contractile activity of skeletal muscle.

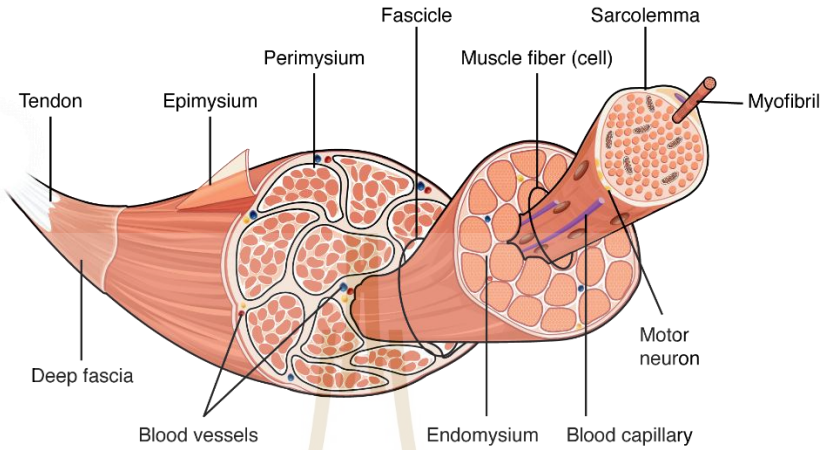

Figure 2.1 The basic structure of skeletal muscle (Biga et al., 2020).

2.2 Skeletal muscle development

Skeletal muscle formation is a complicated process in vertebrates. Skeletal muscle growth mainly depends on the proliferation and differentiation of myogenic cells pre-hatch and accumulation of satellite cells post-hatch.

Muscle fibers are the basic structural element of skeletal muscle which is made up of multinuclear fibers organized in parallel arrays, every fiber is generated through fusion that come from specialized mononuclear cells (myoblasts) (Wigmore and Dunglison, 2002). During the embryonic period, skeletal muscle growth is originated from paraxial mesoderm, properly speaking, from the dermomyotome (Picard et al., 2002), and then produce the myoblasts. In other words, myoblasts are grown by myogenic precursor cells of mesoderm, exiting the cell cycle and cease dividing then continue to differentiate via special signal (Rehfeldt et al., 2000). Then myoblasts

undergoes proliferation, migration, adhesion and differentiate into post mitotic myocytes, finally, fusing to form a multinucleated myotube (Figure 2.2) (Knight and Kothary, 2011). During the development from myotubes to muscle fibers, the first set of muscle fibers in embryo is named as primary fibers, which centrally located nuclei, mononucleated myogenic cells around the primary fibers that will differentiate into secondary fibers. Then Z band of the sarcomere and the muscle contractile unit will arrange and form mature muscle fiber (Figure 2.3) (Swartz, 1994; Velleman, 2007).

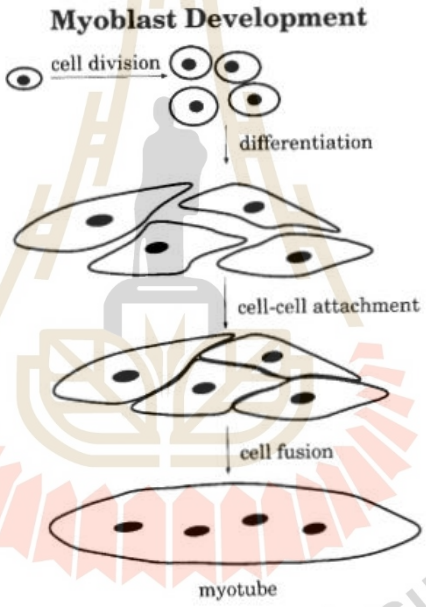

Figure 2.2 The process of myotube development from cells (Swartz, 1994).

Skeletal muscle growth is completed in two period, the muscle cell number is fixed at hatch, called hyperplasia. Whereas, hypertrophy refers to the increase of muscle cell size after hatch (Figure 2.4).

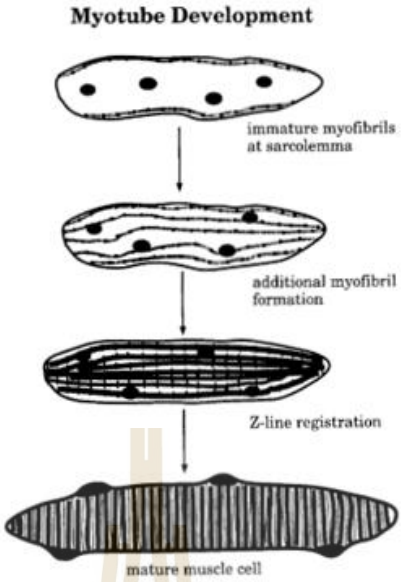

Figure 2.3 The process of mature muscle fiber from myotube (Swartz, 1994).

During post hatch period, the increase of skeletal muscle mass mainly depends on the increase of muscle size, that is the increase of protein deposition, which is the result of transcription and translation of more DNA (Velleman, 2007). In order to acquire more DNA, the increase of nuclei number is required. Another type of cells, is termed satellite cells, which are myogenic precursor cells that are between the sarcolemma and the basal lamina of myofiber (Mauro, 1961), then myonuclear accretion occurs when satellite cells bind to growing muscle fibers (Moss and Leblond, 1971), in this case, causing the increase of muscle fiber size.

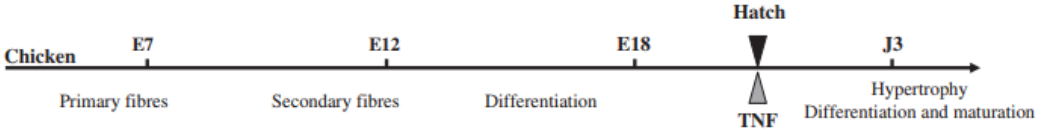

Figure 2.4 The formation of primary fibers and secondary fibers (Picard et al., 2010).

2.3 Genetic regulation of skeletal muscle development

Skeletal muscle development need proliferation of progenitor cells and myogenesis development. Muscle progenitors contains embryonic muscle differentiation then turn into myoblasts. Some of progenitors turn into satellite cells in postnatal muscle, and form committed cells. Eventually activated satellite cell go back to the quiescent satellite cells (Bentzinger et al., 2012). The process of transcription factor regulating progression as shown in Figure 2.5. The dermomyotome includes the reservoir of progenitors cells in proliferative skeletal muscle (Parker et al., 2003). The paired-box transcription factor, Pax3 and Pax7 are important markers of these cells. Pax3 is important for the formation and specification of myogenic cells during embryonic development. Pax7 is an important regulator of satellite cell (Oustanina et al., 2004). Lack of Pax7 does not influence the muscle formation, but the muscle growth will reduced postnatal in mice (Seale et al., 2004). So, Pax3 and Pax7 are upstream factors for myogenic regulatory genes. Myogenesis is a process of muscle development that permit differentiation of mesenchymal cells into myoblasts then exit from cell cycle, which starts to proliferate, fuse together to perform polynuclear syncytia expression in muscle tissue (Musumeci et al., 2015). Myogenic regulatory factors (MRFs) are important markers, that specify the development lineage of the muscle fiber, ensuring the initiation of differentiation programme (Nesvadbova and Borilova, 2018). MRFs is driven by MyoD, Myf5, MyoG, MRF4 (Weintraub et al., 1991) and are specific basic helix-loop-helix transcription factors. MRFs has a conserved domain mediates DNA binding domain, due to the helix-loop-helix motif is required for E-proteins, that mediates the recognition of E-boxes of the promoters of muscle-specific genes (Massari and Murre, 2000). Myf5 and MyoD participate in

muscle specification and trigger conversion in non-muscle cells, such as fibroblasts proliferate to the muscle (Choi et al., 1990). Whereas MyoG and MRF4 regulate terminal differentiation through initiating transcription of genes encoding and allow myotube formation and maturation (Megeney and Rudnicki, 1995; Rudnicki and Jaenisch, 1995; Gerhart et al., 2006). In birds, Myf5 proliferates progenitors, while MyoD presents differentiation in muscle, indicating Myf5 might be expressed before MyoD, Myf5 plays important role in proliferation and maintenance of myogenic-lineage specification before differentiation (Delfini et al., 2000). On the contrary, the MRF4 and MyoG of differentiation factors are not participated in satellite cells development or maintenance (Gayraud-Morel et al., 2007). It has been proved that the absence of MyoG prevented the myoblasts of skeletal muscle, that indicates MyoG induction is indispensable and sufficient for the formation process from myotubes and to fibers (Knapp et al., 2006).

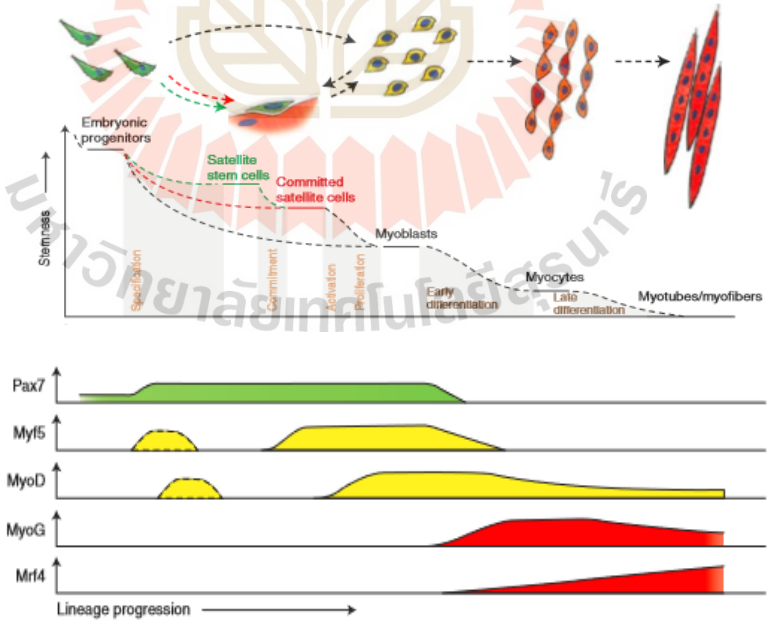

Figure 2.5 Schematic of transcription factors regulating progression in muscle development (Bentzinger et al., 2012).

2.4 Arginine (Arg)

2.4.1 The structure and physicochemical properties of Arg

Arg is one of 20 amino acids needed by animals. In nature, Arg is L-shaped, also named L-arginine (GDR et al., 1984). The molecular formula is $C_6H_{14}N_4O_2$, the molecular weight is 174.20. Hydrochloride of Arg is colorless or white crystalline, airless smell. It includes α-amino group and α-carboxylic acid group, 3-carbon aliphatic straight chain ending of guanidino group make up a side chain. The carboxylic acid is deprotonated ($-COO^-$) and amino group is protonated ($-NH_3^+$) when physiological pH condition is 10.5-12.5. Also, the guanidino group is protonated to form guanidinium form ($-C-(NH_2)_2^+$), which make Arg get charge and to be aliphatic amino acid (Figure 2.6).

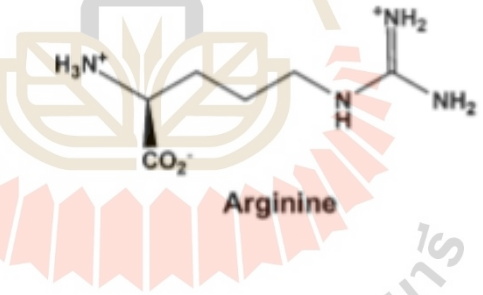

Figure 2.6 Molecule structures of Arg (Morris Jr, 2009).

2.4.2 Arg metabolism

Arg, is essential for poultry, young mammals or animals that is in a state of starvation, trauma, and rapid growth. Due to poultry cannot obtain Arg from endogenous sources, that lack enzymes that are related to the urea cycle (Tamir and Ratner, 1963) , the exogenous supply is necessary in order to meet the needs of nutrition. Arg are precursors of many bioactive molecules, such as, protein, creatine,

ornithine, nitric oxide (NO), glutamate, polyamines, proline, glutamine, agmatine and dimethylargininesa, all of them play important role in biology and physiology (Khajali and Wideman, 2010). The pathways of Arg metabolism are shown in Figure 2.7: (1) Arg synthesize protein directly by mTOR signaling pathway. (2) Arg is catalyzed by nitric oxide synthase to produce citrulline and nitric oxide, which generates nitrous acid and nitrite, then enters the liver and turns into non-toxic nitrogen substances, finally is eliminated from the body. This pathway is the main metabolic pathway of Arg. (3) Arg produces creatine by the action of arginine decarboxylase and further generates phosphocreatine. (4) Arg generates agmatine through the arginine-glycine amidinotransferase. (5) Arg gnerates ornithine and urea by arginase, and ornithine continues to produce pyrolin-5-carboxylase, which further generates proline and glutamate. In poultry, Arg is degraded into ammonia through the ornithine cycle, which produces purines, then decompose to uric acid and is released out of the body. Thereby it plays a vital role in biological and physiological functions (Khajali and Wideman, 2010).

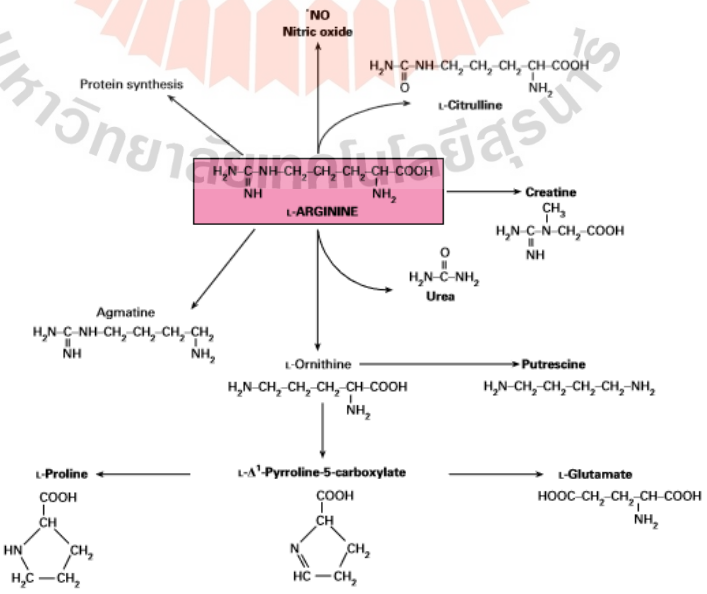

Figure 2.7 The pathway of Arg metabolism (Wu and Morris, 1998).

2.4.3 The action mechanism of Arg in protein synthesis of skeletal muscle

The mechanistic target of rapamycin (mTOR), also named as the mammalian target of rapamycin is a protein kinase has two forms, including mTOR1 and mTOR2. Only mTOR1 can be control by nutrients, such as, amino acid or glucose (Drummond et al., 2011; Kim and Guan, 2011). The mTOR1 with kinase activity plays a very important role in protein synthesis.

Arg is often used in the biosynthesis of protein and cell proliferation. Muscle mass is decided by protein synthesis and breakdown. When the rate of protein synthesis exceeds the rate of protein breakdown that promotes the cell proliferation. The mTOR1 signaling pathway induces the protein synthesis by regulating mRNA translation that are phosphorylated ribosomal protein S6 Kinase (S6k1) and eukaryotic initiation factor 4E-binding protein (4E-BP1) (Ma and Blenis, 2009). The process by Arg induces protein synthesis are shown in Figure 2.8 and 2.9 (Moro et al., 2016). Arg enters to the muscle cell via functional AA transporters that cross the endothelial cell and interstitial space from the blood. When Arg binds to CASTOR1 then disrupts the GATOR2-CASTOR1 complex, permitting GATOR2 to prevent GATOR1 activity. Due to GATOR1, is a complex of three proteins, acting as GAP (GTPase activating protein) for RagA/B, which can inhibit the activation of mTOR1. GATOR2 is located in the upstream of GATOR1, and has the ability to prevent GATOR1, so it has positive effect to regulate the mTOR1. And linking to Rag GTPases that contains RagA or RagB bound to RagC or RagD, that is in response to amino acids in the cell through changing their nucleotide state and their interaction with Regulator. SLC38A9 is putative lysosomal Arg sensor and forms a super-complex with Ragulator, the lysosomal AA transporter links to sensing Arg sufficiency in the lysosome, then interacts with Regulator to activate mTOR1 finally (Wang et al., 2015; Chantranupong et al., 2016).

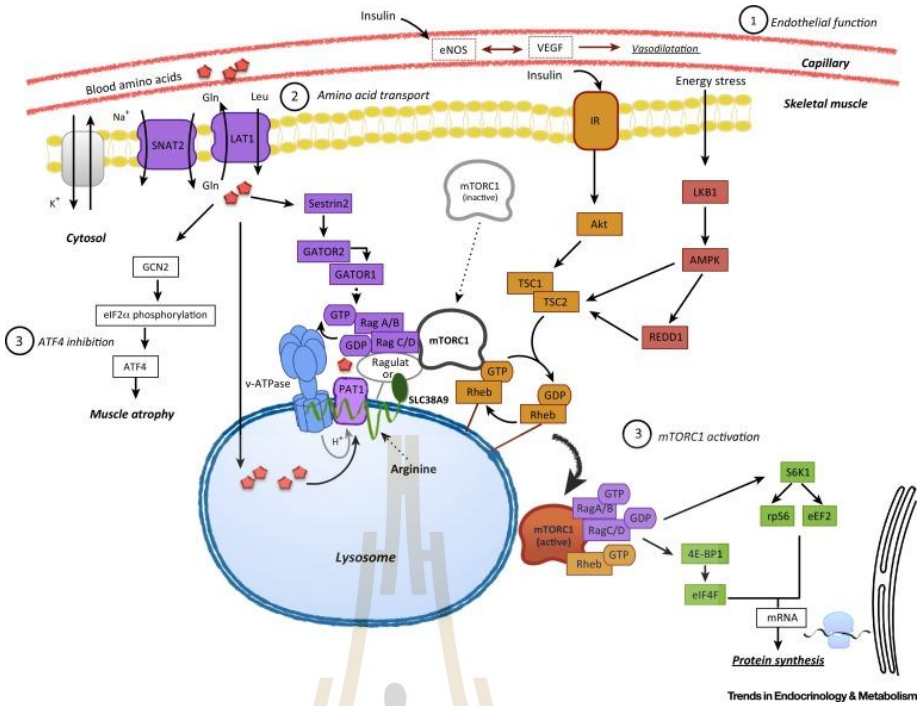

Figure 2.8 Schematic of activating protein synthesis (Moro et al., 2016).

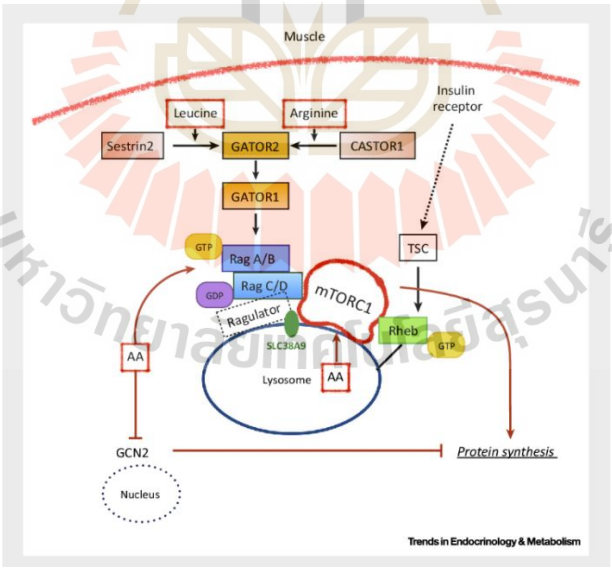

Figure 2.9 Schematic of Arg sensing mechanisms in skeletal muscle fibers (Moro et al., 2016).

2.5 Effects of Arg on poultry production

2.5.1 Effects of Arg on growth performance in poultry

Poultry has an unique and highest requirement for Arg when compared to the mammals (Ball et al., 2007). Multiple functions of Arg have been attracted more interest of many researchers. There are some reports that confirmed at the effects of Arg on growth performance of poultry (Table 2.1). Yu et al. (2018a) reported that the dietary supplementation with 1.19, 1.44, 1.69, 1.94 and 2.19% Arg in the diet, BW was increased in the group of 1.44% Arg and then decreased from 1.69 to 2.19% of Arg in the growth stage (from 14 to 42 days) of layer chickens. Khajali et al. (2011) indicated that supplementing the canola meal diet with 0.4% Arg in broiler chickens diet significantly improved the FI and BWG from 3 to 6 and 1 to 6 weeks of age in feeding period, but no effect on carcass yield, liver, breast and thigh meat yield. Deng et al. (2005) demonstrated that supplementing with 2.7 or 5.4 g/kg Arg in the diet of Leghorn-type chickens significantly increased the BWG and FI, but, the thymus, spleen and bursa were not be affected from 0 to 4 weeks of age. Ebrahimi et al. (2014) reported that adding digestible Arg of 100, 153, 168 and 183% to diets of broiler chickens notably improved the BW, ADBW and feed efficiency on 10, 24 and 46 days respectively. Fernandes et al. (2009) indicated that live weight, carcass weight, breast yield, thigh and leg yield were not be affected from 1 to 21 days in broiler chickens by supplementation with digestible Arg at the levels of 1.30 (control group), 1.49, 1.59, 1.69 and 1.79%. Wu et al. (2011) presented that BWG and breast muscle weight of meat-type ducks was significant increased from 21 to 42 days by adding 1%

Arg (10 g/kg) in the diet. Similar results were found in pigs, dietary 1% Arg supplementation to the diet of growing-finishing pigs significantly increased the

ADG and total skeletal muscle (Tan et al., 2009). In low-birth-weight piglets, dietary supplementation with 0.5% or 1% Arg in the diet for 21 days, the improvement of final BW and ADG were observed respectively (Zheng et al., 2018). On the contrary, 1% Arg did not change the growth performance (ADG, ADFI and F:G) in finishing pigs. According to these previous studies, we make conclusion that Arg has positive effect to improve the growth performance and carcass traits at suitable concentration.

Table 2.1 The effects of Arg on growth performance and carcass traits in poultry.

Poultry	**Dietary Arg supplementation**	**Age**	**Results**	**References**
Layer chickens	1.19, 1.44, 1.69, 1.94 and 2.19%	1 to 42 days	Increasing the BW at 1.44% of Arg	Yu et al. (2018a)
Broiler chickens	0.4%	1 to 6 weeks	Improving the FI and BWG. No effect on carcass yield, breast and thigh muscle yield, and liver weight.	Khajali et al. (2011)
Leghorn-type chickens	2.7 or 5.4 g/kg	0 to 4 weeks	Increasing the BWG and FI. Thymus, spleen and bursa were not be affected.	Deng et al. (2005)
Broiler chickens	100, 153, 168 and 183%	0 to 46 days	Improving the BW, ADBW and feed efficiency.	Ebrahimi et al. (2014)
Broiler chickens	1.30, 1.49, 1.59, 1.69 and 1.79%	1 to 21 days	Live weight, carcass weight, breast yield, thigh and leg yield were not be affected.	Fernandes et al. (2009)
Meat-type ducks	1%	21 to 42 days	Increasing the BWG and breast muscle weight.	Wu et al., (2011)

BW = body weight; ADBW = average daily weight gain; BWG = body weight gain; FI = feed

2.5.2 Effects of Arg on antioxidant capacity in poultry

In order to improve the healthy growth of poultry, an antioxidant system is critical to prevent from free radical production and cell damages (Maritim et al., 2003). Antioxidant includes enzymatic (superoxide, catalase and glutathione reductase, etc) and non-enzymatic agents (some vitamins, minerals and AA). It is well known that one of these functions of Arg is to improve antioxidant capacity, reduce free radical release (Petrović et al., 2008). Duan et al. (2015) reported that supplementation with different levels of digestible Arg (0.96, 1.16, 1.36, 1.56, and 1.76%) in late laying period (61 to 67 weeks of age) had significant effect on MDA and T-AOC levels in broiler breeder serum, egg yolk, serum, liver and breast muscle of one-day-old chicks, and GSH-PX activity were increased in egg yolk, serum, tissues of hatched chicks. Atakisi et al. (2009) indicated that dietary fed with 5mg/kg of Arg to Japanese quails for 30 days significantly decreased MDA contents and increased T-AOC levels of blood. In addition, Xiao et al. (2016) reported that 1% Arg in rats diet significantly improved the levels of T-AOC, GSH and CAT, MDA contents was reduced in jejunum for 30 days. Ma et al. (2010) found that supplementing 0.5 or 1% of Arg to the diet of growing-finishing pigs enhanced T-AOC levels and GSH-PX activity of serum. As for muscle, the T-AOC level was increased and hydroxyl radical was decreased at the concentration of 1% Arg from 90 to 170 days. On the contrary, weanling piglets were fed with 1% Arg did not affect on the antioxidant capacity (Bergeron et al., 2017). In human, the antioxidant capacity of Arg also presented excellent results (Chen et al., 2010). The results of effects of Arg on antioxidant capacity in poultry are summarized in Table 2.2.

Table 2.2 The effects of Arg on antioxidant capacity in poultry.

Poultry	Dietary Arg supplementation	Age	Results	References
Laying hens	0.96, 1.16, 1.36, 1.56, and 1.76%	61 to 67 weeks	T-AOC levels were increased, MDA contents were reduced in broiler breeder serum, egg yolk, serum, liver and breast muscle of hatched chicks; GSH-PX activity were increased in egg yolk, serum, tissues of hatched chicks.	Duan et al. (2015)
Japanese quails	5 mg/kg	30 days	Decreasing MDA contents and increasing T-AOC activity of blood.	Atakisi et al. (2009)

2.5.3 Effects of Arg on genes expression related to the muscle development in poultry

AAs are major regulators of protein metabolism, Arg could regulate protein synthesis and animal growth by activating mTOR signaling pathway, its downstream are S6K1 and 4EBP1 lead to mRNA translation and protein synthesis (Wu et al., 2010). Previous studies proved that function of protein synthesis of Arg by inducing mTOR signaling pathway. Yuan et al. (2015) reported that in vitro experiment, intestinal epithelial cells were cultured in medium included different levels of Arg (100, 200, 400, and 600 μM), the results showed that the mRNA abundances of mTOR, S6K1 and 4EBP1 were significantly upregulated as well as protein concentration were increased. Similarly, in pigs, Yao et al. (2008) demonstrated that neonatal pigs were fed with 0.6% Arg markedly increased the phosphorylation of 4EBP1 and mTOR in longissimus muscle, but no effect on S6K1

phosphorylation. However, the levels of phosphorylated 4EBP1 and mTOR did not change in liver. In vitro experiment, adding different levels of Arg significantly up-regulated the mRNA expressions of mTOR, S6K1 and 4EBP1 of mammary epithelial cells in cattles (Wang et al., 2014).

Moreover, myogenesis of skeletal muscle contribute to regulation of genes. Li et al. (2016) demonstrated that Arg did not affect on the mRNA levels of Pax7 and MyoD. But, mRNA expression of MyoG was increased in vitro experiment (Pender et al., 2017). Madsen et al. (2017) reported that adding Arg in gestation diets did not affect on the mRNA expression of myogenesis genes of newborn pigs. Some results are showed in Table 2.3.

Table 2.3 The effects of Arg on genes expression related with muscle development in poultry

Poultry	Arg supplementation	Age	Results	References
Embryo of broiler chickens	Cell culture, 0.01 or 0.05 mM	Embryonic 14th days	mRNA levels of Pax7 and MyoD were not changed, but, mRNA expression of MyoG was increased.	Li et al. (2016)
Embryo of broiler chickens	Cell culture, 100, 200, 400, and 600 μM	Embryonic 14th days	mRNA abundances of mTOR, S6K1 and4EBP1 were significantly increased as well as protein concentration were increased.	Yuan et al. (2015)

2.6 In ovo feeding technology

In order to improve the chicken growth after hatch, a methodology that is in ovo feeding (IOF) was developed, which is the nutrients solution are injected into embryo of poultry. The first administration of in ovo delivery of external nutrition was operated in the early 1980s for Marek's disease (Sharma and Burmester, 1982). That is a successful technique and the definition have been defined. IOF (Uni and Ferket, 2003), that is, injecting nutrients into amnion of embryo, due to the birds naturally consumers the amniotic fluid (main water and albumen protein) before pipping of the air cell. So, supplementing nutrients into the amnion is fundamentally feeding the embryo an external diet prior to hatch.

On the other hand, the development period of embryo and injection time play an important role for the maximum hatchability and chick quality (Kadam et al., 2013). In ovo technology includes different substances, which can be injected into the air chamber, yolk sac or amnion with developing embryo. Different location is injected nutrient that is decided by different age of embryo. In fresh egg or the primary phase of embryonic development, components are managed to the egg protein at 12 mm, which allows the specification of comoponent as near to the germinal disc as possible. In the later development phase of embryo, nutrients are often injected to the yolk sac. After 17 days of embryonic develoment, The main area of the egg for injection nutrition is the amnion or air chamber (Ebrahimi et al., 2012).

IOF is expected to some advantages, these nutrients support the chicken growth, reduce the mortality and morbidity post-hatch then improve the chicken quality (Noy and Uni, 2010), such as, development of skeletal muscle and immune system. Therefore, IOF plays an important role and which is a tool to overcome early

growth constraints during embryonic and post-hatch development in poultry (Foye et al., 2006b).

2.6.1 In ovo feeding of nutrients for poultry growth

With the development of in ovo technique, many bioactive substances are injected directly to embryo. Such as, amino acids, carbohydrates, vitamins and minerals. This technique compensates for starvation period of chickens and facilitates gastrointestinal tract health, muscle growth and development of antioxidant and immune systems. Finally, improving chicken performance post-hatch. In the past few decades, the accomplishments are tremendous in the field of in ovo nutrition, which are summarized as follows: Gao et al. (2018) reported that IOF of 1% Arg had significant effect on FI, BWG and small intestine functions from 1 to 42 days post-hatch. And, some genes and protein abundances of mTOR, S6K1 and 4EBP1 were positively affected in jejunum of broiler chickens on 21 days post-hatch. Similarly, Zhang et al. (2017) demonstrated that BW and BWG, weights of carcass, breast muscle and some organs were affected during the period of DOH to D14 post-hatch by IOF of 1% Arg in pigeons. Yu et al. (2018b) found that relative mRNA expression of mTOR was enhanced on D3, D7 and D21 post-hatch respectively, whereas relative mRNA expressions of S6K1and 4EBP1 were increased as well as total protein on D3 and D21 post-hatch by IOF of 1% Arg to broiler chickens. And, Al-Daraji et al. (2012) demonstrated that injected 1, 2 and 3% of Arg in embryo resulted in significant improvement in BW, BWG and some organs weight. Meanwhile, FCR, backbone, wings and neck were decreased on 42 days in Japanese quail. Subramaniyan et al. (2019) found that protein expressions of MyoG and MyoD were significantly up-

regulated, whereas the hatchability, immune system and BW were improved on embryonic 14th day by IOF of Arg.

Dong et al. (2013) reported that IOF of 2.5% maltose + 2.5% sucrose on embronic 14.5 days improved the hatchability, BW and pectoral muscle weight that may by increasing the pectoral muscle glycogen reserves on hatch day in pigeons. Chen et al. (2009) demonstrated that IOF of maltose, sucrose, and glutamine into amniotic fluid on embryonic 23 days, improving small intestine development and pectoralis weight in ducks. Tako et al. (2004) found that the villi size of small intestine and BW were increased by IOF of carbohydrates and β-Hydroxy-β-Methylbutyrate on D17.5 of incubation in broiler chickens. Other researchers also stated that IOF of carbohydrates had positive effect on glycogen reserves and chicken growth (Foye et al., 2006a; Tangara et al., 2010; Zhai et al., 2011). Moreover, according the report of Zhao et al. (2017), IOF of CrPyr (12 mg/egg) at D17.5 of incubation increased mRNA expressions of MyOD, MyOG, and Pax7, morphology traits, and satellite cell activity then improved breast muscle growth in late embryos and neonatal broiler chickens. Moreira Filho et al. (2019) reported that IOF of different levels of threonine (0, 1.75, 3.50, 5.25 and 7.00%) on D17.5 of incubation enhanced the growth performance, morphology traits and mRNA expressions of small intestine on DOH and D21 post-hatch in broiler chickens. Elwan et al. (2019) also stated that levels of T-AOC and GSH and morphology traits were significantly increased, at the same time, gene expression of heart, muscle, jejunum and liver were up-regulated on DOH by IOF of methionine-cysteine in broiler chickens. Xu et al. (2019) reported that IOF of histidine on D13 of incubation had beneficial effect on hatchability and some organs development post-hatch in pigeons. On the contrary, Zhu et al. (2019) found that IOF of lysine on D13

of incubation had negative effects on hatchability and morphology traits post-hatch in pigeons. Moreover, Mroczek‐Sosnowska et al. (2016) demonstrated that IOF of nano-copper or copper sulfate on D1 of incubation decreased the mortality, positively effected on the growth performance, percentage of breast and leg muscle on D42 post-hatch in broiler chickens. Oliveira et al. (2015b) reported that IOF of minerals (Ca, P, Mg, Mn and Zn) had potential functions to improve bone mineralization. The similar results were found by Oliveira et al. (2015a) and Bello et al. (2014). Also, IOF of vitamins influenced on the poultry quality. Fatemi et al. (2020) demonstrated that FI and FCR were decreased on D14 post-hatch in broiler chickens by IOF of vitamin D3 or 25-hydroxylcholecalciferol on D18 of incubation, which means there was a potential effect to improve growth performance. Zhang et al. (2019) stated that IOF of ascorbic acid (3, 6, 12 or 36 mg) on D17 of incubation had lasting positive effects on growth performance, antioxidant capacity and leg muscle development on D45 post-hatch in broiler chickens, meanwhile, higher dosages of ascorbic acid may increase meat quality. Recent years, the probiotics or synbiotics are getting more attention around the world, Pender et al. (2017) indicated that IOF of probiotics on D18 of embryo improved the BW and BWG at the first week post-hatch, upregulated the gene expressions of ileum and cecal during 0 to 22 days in broiler chickens. Tavaniello et al. (2019) reported that in ovo injection of different synbiotics on embroynic 12th day showed positive effect on nutritional traits in meat, but did not affect on the carcass traits during 0 to 42 days in broiler chickens. Pruszynska-Oszmalek et al. (2015) demonstrated that IOF of prebiotics and synbiotics on 12th day increased final BW on D34 post-hatch in brioler chickens. Some results of IOF of nutrients are showed in Table 2.4.

Table. 2.4 The effects of IOF of nutrients for poultry growth.

Poultry	Injected substances	Injection time	Age	Results	References
Broiler chickens	1% Arg	E17.5	D1 to D42	Increasing the FI, BWG, and small intestine functions. some genes, protein of mTOR signaling pathhway were affected.	Gao et al. (2018)
Broiler chickens	CrPyr (12 mg/egg)	E17.5	E19 to D7	Increasing mRNA expressions of MyOD, MyOG, and Pax7, morphology traits, satellite cell activity and breast muscle weight.	Zhao et al. (2017)
Broiler chickens	Different levels of threonine (0, 1.75, 3.5, 5.25 and 7%)	E17.5	DOH to D21	Enhancing the growth performance, morphology traits and mRNA expressions of small intestine.	Moreira Filho et al. (2019)
Broiler chickens	Vitamin D3 or 25-hydroxylcholecalciferol	E18	0 to 14 days	FI and FCR were decreased.	Fatemi et al. (2020)
Broiler chickens	Ascorbic acid (3, 6, 12 or 36 mg)	E17	0 to 45 days	Having lasting positive effects on growth performance, antioxidant capacity and leg muscle development.	Zhang et al. (2019)
Broiler chickens	Different probiotics	E18	1 to 22 days	Increasing the BW and BWG, up-regulated the gene expression of ileum and cecal post-hatch.	Pender et al. (2017)
Broiler chickens	Different synbiotics	E12	0 to 42 days	Positively affect on nutritional traits in meat, but did not affect on the carcass traits.	Tavaniello et al. (2019)

E=embryo; BW= body weight; BWG=body; FI=feed intake; FCR=feed conversion ratio.

Taken together, in ovo technique is a possible way to get the best results for enhancing the productivity. Different nutrients that injected to the different bird have different effects, which depends on the volume and concentration of the nutrients. However, limitations of this technique shoud be considered about the appropriate sites and age of injection for better outcome that is a systematic work should be explored in the furture work, which could be practically benefit for the poultry production.

2.7 Korat chicken

"Korat chicken", slow-growing chicken, is Thai indigenous crossbreed strain that comes from male Thai indigenous chickens (Leung Hang Khao) and female Suranaree University of Technology (SUT) breeder line. Body weight of Korat chicken reached 1.2 kg during 63 days of age. Their meat has less fat and more collagen (Sangsawad et al., 2016). So, it has firmer and chewier texture and the price is 1.5-2 times higher than that of broiler meat.

The demand of indigenous chickens is increasing. In Southeast Asia, such as, Thailand, indigenous chickens are often favored broadly by consumers. Korat chickens have better growth performance than other indigenous chickens. Production of Korat chickens steadily increases and has a potential to be one of community livestock. By far, there are some studies have been published to improve the productivity of Korat chickens (Hang et al., 2018; Kubota et al., 2019; Maliwan et al., 2019; Katemala et al., 2021). However, the requirement of Arg could not be obtained in Korat chickens, Arg requirement of broiler chicken are 1.25% and 1.10% on 0 to 3 weeks and 3-6 weeks respectively by NRC (1994) recommendation that can be still

referenced by Korat chicken, which is useful information to explor the effect of Arg (dietary or in ovo feeding) on Korat chickens. In order to expend the raising of Korat chickens by farmers to improve their incomes and market share. It should be paid more attention to continue to study in the future.

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CHAPTER III

MATERIALS AND METHODS

3.1 Materials and methods

3.1.1 Eggs and incubation

Fertile eggs were obtained from SUT hatchery (Nakhon Ratchasima, Thailand). All eggs were weighed and randomly allocated into incubators, the incubating condition were maintained at the temperature of 37.5℃ with 60% relative humidity and eggs were turned automatically per hour. On day 14 of embryo, all eggs were checked by electric torch, those unfertilized and nonviable eggs were removed and discarded. Meanwhile, total 480 embryonated eggs with similar weight (59.0±1.0 g) were randomly divided to 2 treatment groups, each treatment contains 4 replicates of 60 eggs each. Each treatment was placed on two trays and 4 trays were used totally in this experiment.

3.1.2 Preparation of solution and in ovo feeding procedure

Arg solution was prepared by using 0.9% saline (A.N.B Laboratories Co., Ltd., Bangkok, Thailand). 1% Arg was used in this study, that is, 10 g Arg (Sigma-Aldrich Inc., St Louis, MO, USA) was dissolved in 1 L saline of 0.9%, it was equivalent to 5 mg of Arg per egg, this concentration of Arg was selected according to previous studies (Gao et al., 2017; Yu et al., 2018) with some modification. The Arg solution was sterilized by autoclaving at 120℃ for 15 min, then was filtered through 0.45 μm membrane filter.

IOF of Arg was performed on day 18 of incubation, all eggs were taken out from incubators, 2 of 4 trays were non-injected group, considered as control group, other 2 trays were injected 1% Arg, as Arg group. The eggs were examined to make sure the live embryos, the large end surface of eggs were disinfected by 75% alcohol, the pinhole was punched and 0.5 ml of Arg solution was injected into amniotic sac using 21-gauge needle (Uni et al., 2005). The eggs of non-injected control group were kept outside the incubators to have the same environmental condition as Arg group. The IOF progress was completed in 2 hours. After injection, the holes were sealed by paraffin wax, and then were placed to the hatching baskets. The eggs from each group were randomly divided into 4 replicates (60 eggs/replicate), each basket was viewed as a replicate. The eggs of 8 baskets were transferred to hatcher and continued to perform the hatchery program.

3.1.3 Birds rearing

On day of hatch (DOH), the number and the weight of hatched birds were recorded, the birds of each treatment were pooled and sexed by anus identification. 160 mixed-sex and healthy birds of each treatment with similar BW that near to the mean BW of the pooled group, then were randomly divided into 4 replicates of 40 birds each. Birds were raised in 8 litter floor pens (replicates) of open-sided house with natural ventilation. All birds were obtained ad libitum the commercial feed (Charoen Pokphand Co., Ltd., Nakhon Ratchasima, Thailand) and fresh water, the nutrient contents of feed for three feeding stages of 63 days were analyzed, the nutrient compositions of the basal diet is shown in Table 3.1. The vaccination program of birds was followed the guidelines of SUT farm. The BW and feed intake on a pen basis were recorded weekly, then BWG and FCR were calculated on their pen basis.

Table 3.1 Nutrient compositions of the basal diet.

Item	Starter diet 0-21 d	Grower diet 22-42 d	Finisher diet 43-63 d
Analyzed composition (%)			
Dry matter	93.83	93.51	94.21
Gross energy (MJ/Kg)	12.54	12.96	13.38
Crude protein	22.72	20.46	18.65
Crude fat	5.20	6.74	6.66
Crude fiber	3.44	3.45	3.55
Crude ash	4.70	4.58	4.19
Lysine	1.78	1.43	0.92
Methionine	0.34	0.25	0.28
Threonine	1.01	0.85	0.73
Arginine	1.58	1.13	0.55

3.1.4 Tissue collection

On DOH, three male chickens per replicate with similar BW near to the average BW of their cages, were chosen and weighed on DOH. The breast muscle of two chickens were obtained and weighed after killing by chloroform, the muscle tissue were frozen in liquid nitrogen and stored at -80°C for further analysis. The breast muscle tissue of the third chickens was obtained and fixed in 4% paraformaldehyde for morphological analysis.

On D21 and D42 post-hatch, two male chickens were selected from per replicate of two groups respectively, chickens were sacrificed and collected the breast muscle into liquid nitrogen and 4% paraformaldehyde for further analysis. On D63,

two male chickens per replicate were randomly selected that BW were near to the average BW of their cage. The breast muscle of one chicken was obtained and weighed after killing by chloroform, the breast muscle tissue was frozen in liquid nitrogen for further analysis. The small parts of breast muscle was fixed in 4% paraformaldehyde for morphological analysis. Another chicken was electrically stunned and slaughtered. Carcasses were blast chilled at 4°C for 24 h. After chilling, carcasses were eviscerated and weighed to make sure the dressing percentage. The abdominal fat, liver, heart and gizzard were removed to calculate their percentage based on live BW. Eviscerated carcass percentage were calculated based on the live BW. The entire right breast muscles was measured for meat quality, while the left side of breast muscles were determined for chemical composition.

3.1.5 Sample measurement

Chemical compositions: The proximate compositions included dry matter, crude protein and crude fat contents were estimated by official methods of AOAC (1990). Fresh samples were dried at 60°C for 72 h prior to the measurement. After that, the dry matter of samples was analyzed by muffle furnace (Carbolite Gero, GPC 1200, Derbyshire, UK). The samples were ground to perform the crude protein and crude fat. Crude protein content was measured from 0.5 g dried meat using Combustion Nitrogen Analysis (Elementar Analysensysteme GmbH, Hanau, Germany). Crude fat content was measured by petroleum ether extraction using Soxtet 8000 extraction system (Foss Analytical Co., Suzhou, China).

Morphological observation: The sections (10 um) of breast muscle were cut and put them on glass slices, stained with hematoxylin and eosin. All sections of breast muscle were observed by microscope equipment and the five

sections per sample were analyzed. The numbers and diameters of breast muscle fiber were analyzed by image analyzer (Image Pro Plus 5.0 software, Media Cybernetics Inc., Bethesda, MD, USA).

Meat quality: Meat quality were measured by the parameters of meat pH, color, shear force, drop loss and cooking loss. Muscle pH was determined using hand-held digital pH meter (Ultra Basic pH meter, Model UB10A, Denver Instrument, Bohemia, NY, USA) at 45 min and 24 h of postmortem on breast muscle. The muscle

color included lightness (L*), redness (a*), and yellowness (b*) were detected by chroma meter on breast muscle after 24 h of postmortem. Drip loss was measured follow Zhang et al. (2017) with some modification. Briefly, the size of 3×2 ×1 cm samples were cut from breast muscle, weighted and placed in plastic bag filled with air. All samples were suspended in refrigerator at 4°C. After 24 h, samples were wiped with gauze and reweighted again. Drip loss percentage was calculated as follows: (initial weight-final weight)/initial weight×100. The method of cooking loss and shear force were followed by Cong et al. (2017) with some modification. After 24 h of postmortem, the breast meat were weighed and put them into zip-sealed plastic bag, cooked in digital water bath kettle at 85°C and the internal temperature was 77°C , then the samples were taken out and cooled to 4°C and wiped with filter paper to remove the moisture and reweighed. Cooking loss percentage was calculated as follow: (initial weight-final weight)/initial weight×100. The shear force were detected by cooked samples that were cut to small strip of 1×1×3 cm using Instron texture system (Model5565, Instron Corporation, Burlington, ON, Canada).

Amino acid analyses: The amino acid compositions were measured by the previous description (Liu et al., 2015) with modification. 80 mg of fresh breast muscle with 4 ml of 6 M hydrochloric acid solution was served in 10 ml hydrolysis glasses and filled with nitrogen gas for 5 min, then transferred to the heating incubator at 110°C for 24 h. After that, the supernatant was moved to the centrifuge tubes through 0.45 μm membrane filters to ready use. Further, the 1 μL solution was prepared for injection, including 40 μL internal standard (norleucine), 250 μL sample

supernatant and 710 μL buffer that were measured using ultra ninhydrin solution at 570 nm of detection wavelengths by amino acid analyzer (Biochrom 30+, Cambridge, UK). The amino acid contents are showed as mg per gram of breast muscle.

Measurement of antioxidant capability

Malondialdehyde (MDA): Lipid peroxidation is a marker of oxidative stress. MDA concentration of breast muscle was measured at 532 nm via the method of thiobarbituric acid (TBA) using lipid peroxidation (MDA) assay kit (Catalog Number MAK085, Sigma-Aldrich, USA). The details of measurements followed the manufacturer's instructions. The results obtained were expressed as nmole of MDA per mg tissue.

Total Antioxidant Capacity (T-AOC): T-AOC was measured by commercially available kit (Catalog Number MAK187, Sigma-Aldrich, USA). In this kit, either the concentration of small molecule binds to the protein antioxidants, or only the concentration of small molecule antioxidants can be measured. Cu^{2+} ion is converted to Cu^{+} through both small molecules and proteins. The reduced Cu^{+} ion chelates with a colorimetric probe, and the absorbance can be detected at 570 nm. The

details of measurements followed the manufacturer's instructions. T-AOC values were expressed as nmole per mg protein.

Glutathione (GSH): The antioxidant capacity of muscle GSH was measured by using commercially available kit (Catalog Number CS0260, Sigma-Aldrich, USA), GSH concentration was quantified through reaction with 5,5'-dithiobis-p-nitrobenzoic acid to produce 5-thio-2-nitrobenzoic acid, which can be detected at 412 nm. The details of measurements followed the manufacturer's instructions. Results was expressed as nmoles GSH per mg tissue.

RNA extraction and cDNA synthesis: Total RNA was extracted from the breast muscle using Trizol Reagent (Invitrogen; Thermo Fisher Scientific) by following the manufacturers'instructions. The RNA quality was checked by 1.0% agarose gel electrophoresis, and the purity and concentration was checked by NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) based on the OD value of 260/280. DNase I was treated to eliminate DNA contamination. Then, cDNA was synthesized by using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems; Thermo Fisher Scientific) according to the instructions of manufacturer.

Real-Time PCR: The primers of target genes were designed by primer Premier 5 software according to the mRNA sequences of Gallus Gallus in NCBI database and synthesized by company (Gibthai Co., Ltd., Bangkok, Thailand). The endogenous reference gene was glyceraldehyde 3-phosphate dehydrogenase (GAPDH) that was recommended (Zhao et al., 2017). The sequences are listed as Table 3.2. The primers of all genes were checked by using routine PCR. The mRNA expression of these genes were quantified by Real-Time PCR using Light Cycler 480 System (Roche Diagnostics GmbH, Mannheim, Germany). The standard curve were

Table 3.2 Primers sequences used for real-time PCR.

Gene	Gene bank accession no.	Primers Sequence (5'-3')	Annealing temperature (°C)	Product size (bp)
Pax7	NM_205065.1	F:CAAAGGGAACAGGCTGGATG	60	102
		R:TGCTCGGCAGTGAAAGTGGT		
MyoD	L34006.1	F:CACAGTCACCGTTTTCCCA	57	102
		R:GCCTCACAGCACAAGCATC'		
Myf5	NM_001030363.1	F:GAGGAGGAGGCTGAAGAAAG	59	119
		R:CGATGTACCTGATGGCGTT		
MyoG	NM_204184.1	F:GGATGGTGATGCTGGAAGGA	57	93
		R:GGAAAGGATTTGGGCGGTT		
MRF4	D10599.1	F:AGGCTCTGAAAAGGCGGACT	59	169
		R:TTGGGGCTGAAGCTGAAGG		
mTOR	XM_417614.6	F:CTCAGCGGGAACCAAAAGA	57	125
		R:ATGGATTCGGTCATCACGG		
4EBP1	XM_424384.6	F:CCTGATGGAGTGCCGTAAT	57	77
		R:GGCTGGTAACACCTGGAAT		
S6K1	NM_001030721.1	F:GAGGAGTGGGCATAATCGTG	58	154
		R:TGTGAGGTAGGGAGGCAAAT		
GAPDH	NM 204305.1	F:GAGGGTAGTGAAGGCTGCTG	60	113
		R:CATCAAAGGTGGAGGAATGG		

Pax7 = paired box 7; MyoD = myogenic differentiation 1; Myf5 = myogenic factor 5; MyoG = myogenin; MRF4 = muscle regulator 4; mTOR = mechanistic target of rapamycin; 4EBP1 = eIF4E-binding protein 1; S6K1 = ribosomal protein S6 kinase 1; GAPDH = glyceraldehyde 3-phosphate dehydrogenase.

performed to check efficiency of all genes before carrying out the Real-time. These reaction system were performed in 20 μL volume, containing 10 μL of SYBR Green Master Mix (Applied Biosystems, Thermo Fisher Scientific), 2 μL of cDNA (diluted 1:10), 1 μL of forward primer (10 μM), 1 μL of reverse primer (10 μM), and 6 μL of diethylpyrocarbonate-treated water. The cycling condition of Real-time PCR were initial denaturation at 95°C for 10 min, 40 cycles of denaturation at 95°C for 30 s, annealing temperature of specific primers for 35 s, and final dissociation stages were at 95°C for 5 s and 72°C for 5 min, the melting curve were analyzed the specificity of all target genes. Each sample of all target genes were carried on in triplicate. The difference of the mRNA expression in each target gene was calculated by using the $2^{-\Delta\Delta ct}$ method (Livak and Schmittgen, 2001).

3.2 Introduction

All data were analyzed by unpaired t-test by using SPSS software (IBM Corp. 1989, 2013. New York, NY), the statistical significances between two groups was denoted at $P<0.05$. The results were expressed as means and SEM. In addition, pearson correlation coefficients were evaluated to determine the relationship between antioxidant capacity and meat quality. Principal component analysis was performed to classify the samples from two treatments for visualising the underlying data structure, and determine the relationship between breast muscle traits and genes expression of muscle development in two groups by using the Unscrambler X 10.5 software (CAMO Software, Oslo, Norway).

3.3 References

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CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Results

4.1.1 Hatchability and growth performance

As shown in Table 4.1, the results of hatchability and growth performance of Korat chickens indicated that there were no significant difference (P> 0.05) in hatchability, BW, BWG, FI and FCR between control and Arg groups.

4.1.2 Carcass traits

The effects of IOF of Arg on carcass traits of Korat chickens are presented in Table 4.2, these results revealed that IOF of Arg did not improve (P> 0.05) carcass traits (dressing yield, eviscerated yield, breast muscle, thigh muscle, abdominal fat, heart, liver and gizzard) compared with the control group.

The effects of IOF of Arg on breast muscle weight are presented in Figure 4.1, the breast muscle weight in Arg group were not increased (P>0.05) on DOH, D21, D42 and D63 post-hatch respectively in Korat chickens compared with the control group.

4.1.3 Muscle fiber traits

In Table 4.3, no significant differences in the index of muscle fiber diameter were observed (P>0.05) between control and Arg groups on DOH, D21, D42 and D63 post-hatch respectively. The muscle fiber number of Korat chickens was not significantly increased (P>0.05) on D63 by IOF of Arg compared with the control group.

Table 4.1 Effects of in ovo feeding of Arg on hatchability and growth performance in Korat chickens.

Items	Treatments		SEM	P value
	Control	Arginine		
Hatchability (%)	86.25	87.09	1.910	0.768
BW (g)				
DOH	44.52	43.99	0.230	0.154
D21	324.73	317.39	2.303	0.091
D42	788.75	775.00	2.423	0.279
D63	1277.12	1227.69	18.275	0.139
BWG (g)				
DOH-21	280.21	273.40	2.394	0.122
D21-42	464.02	457.61	5.271	0.591
D43-63	488.37	452.69	16.085	0.218
DOH-63	1232.60	1183.70	18.360	0.141
FI (g)				
DOH-21	569.78	573.37	25.680	0.924
D21-42	1026.70	1024.82	21.011	0.955
D42-63	1261.64	1233.98	37.722	0.652
D0-63	2858.11	2832.16	35.754	0.655
FCR				
DOH-21	2.03	2.10	0.092	0.581
D21-42	2.21	2.24	0.052	0.794
D42-63	2.58	2.73	0.045	0.084
D0-63	2.32	2.39	0.022	0.071

Values are represented by means±SEM. DOH = day of hatch; BW = body weight; BWG = body weight gain; FI = feed intake; FCR = Feed conversion ratio. Control = Non-injected group. Arg = 1% L-Arginine group.

Table 4.2 Effects of in ovo feeding of Arg on carcass traits in Korat chickens**.**

	Treatments			
Items	**Control**	**Arginine**	**SEM**	**P value**
Dressing yield (%)	79.14	79.67	0.576	0.626
Eviscerated yield (%)	65.36	66.89	0.910	0.320
Abdominal fat (%)	1.35	1.20	0.280	0.812
Heart (%)	1.06	1.09	0.213	0.931
Liver (%)	2.00	1.83	0.100	0.418
Gizzard (%)	2.30	2.23	0.150	0.851

Values are represented by means ± SEM. Control = Non-injected group. Arg = 1% L-Arginine group.

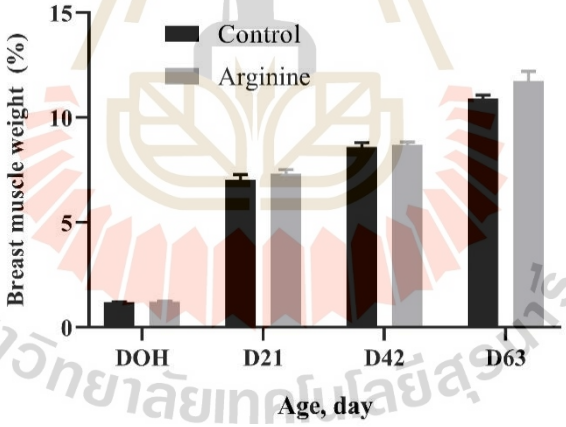


Figure 4.1 Effects of in ovo feeding of Arg on breast muscle weight (absolute weight normalized to BW, %) on DOH, D21, D42 and D63 post-hatch in Korat chickens. Values are represented by means±SEM. Control = Non-injected group. Arg = 1% L-Arginine group.

Table 4.3 In ovo feeding of Arg on muscle fiber diameter and number on breast muscle in Korat chickens.

Items	Treatments		SEM	P value
	Control	Arginine		
Muscle fiber diameter (µm)				
DOH	5.69	5.95	0.138	0.272
D21	18.03	20.11	0.832	0.199
D42	22.78	22.89	0.703	0.924
D63	33.63	34.43	0.216	0.599
Muscle fiber number, D63 (no.100 µm^2)	4.65	4.38	0.135	0.256

Values are represented by means±SEM. Control = Non-injected group. Arg = 1% L-Arginine group.

4.1.4 Amino acid compositions

The compositions of amino acid in breast muscle of different growth periods (DOH, D21, D42 and D63 post-hatch) are presented in Table 4.4 to 4.7. As shown in Table 4.4, some amino acids contents (Ser, Glu, Pro, Gly, Val, Met, Ile, Phe, His, Arg) in breast muscle on DOH were significantly affected (P<0.05) by IOF of Arg compared to the control group.

As shown in Table 4.5, the contents of Pro, Phe and Arg were significantly higher (P<0.05) in breast muscle on D21 by IOF of Arg compared to the control group.

In Table 4.6, IOF of Arg significantly affected (P<0.05) the contents of Val, Ile, His and Arg in breast muscle on D42 when compared to the control group.

Table 4.4 Effects of in ovo feeding of Arg on amino acid compositions in breast muscle on DOH in Korat chickens (mg/g).

Items	Treatments		SEM	P value
	Control	Arginine		
Asp	11.60	12.35	0.280	0.106
Thr	4.54	5.12	0.199	0.085
Ser	4.59[b]	5.40[a]	0.200	0.029
Glu	21.49[b]	23.35[a]	0.422	0.035
Pro	5.59[b]	7.25[a]	0.212	0.001
Gly	17.06[b]	18.65[a]	0.327	0.014
Ala	13.02	14.00	0.367	0.106
Val	11.64[b]	12.61[a]	0.233	0.042
Met	3.49	3.58	0.022	0.050
Ile	7.13[b]	7.73[a]	0.136	0.020
Leu	10.01	10.50	0.196	0.128
Tyr	2.97	3.37	0.170	0.16
Phe	3.89[b]	4.31[a]	0.072	0.012
His	3.47[b]	4.09[a]	0.114	0.016
Lys	11.26	11.95	0.326	0.189
Arg	5.72[b]	7.08[a]	0.275	0.023

Values are represented by means±SEM. [a,b]Means in the same row with different superscript are significantly different at $P<0.05$. Control = Non-injected group. Arg = 1% L-Arginine group.

Table 4.5 Effects of in ovo feeding of Arg on amino acid compositions in breast muscle on D21 in Korat chickens (mg/g).

	Treatments			
Items	**Control**	**Arginine**	**SEM**	**P value**
Asp	26.96	26.75	0.338	0.693
Thr	11.61	12.16	0.415	0.381
Ser	11.00	10.78	0.407	0.714
Glu	42.79	43.58	0.737	0.477
Pro	10.36^{b}	11.87^{a}	0.379	0.030
Gly	25.77	25.24	0.391	0.375
Ala	27.26	26.47	0.609	0.566
Val	24.69	25.57	0.425	0.229
Met	8.29	8.53	0.087	0.132
Ile	17.29	18.23	0.377	0.204
Leu	21.93	22.47	0.374	0.384
Tyr	7.80	7.65	0.081	0.273
Phe	9.40^{b}	9.88^{a}	0.120	0.046
His	21.15	21.65	0.356	0.374
Lys	25.50	25.92	0.415	0.500
Arg	12.39^{b}	13.23^{a}	0.208	0.029

Values are represented by means±SEM ^{a,b} Means in the same row with different superscript are significantly different at $P<0.05$. Control = Non-injected group. Arg = 1% L-Arginine group.

Table 4.6 Effects of in ovo feeding of Arg on amino acid compositions in breast muscle on D42 in Korat chickens (mg/g)

Items	Treatments		SEM	P value
	Control	Arginine		
Asp	19.51	20.90	0.533	0.114
Thr	9.24	9.84	0.315	0.225
Ser	8.19	8.68	0.329	0.329
Glu	31.66	33.38	0.562	0.074
Pro	9.26	9.86	0.447	0.379
Gly	12.80	13.35	0.406	0.381
Ala	15.80	16.60	0.367	0.249
Val	19.79[b]	21.00[a]	0.349	0.049
Met	5.77	6.31	0.160	0.055
Ile	15.32[b]	16.91[a]	0.352	0.048
Leu	22.78	23.97	0.488	0.172
Tyr	8.56	9.00	0.186	0.181
Phe	8.49	9.43	0.288	0.058
His	23.23[b]	25.19[a]	0.268	0.011
Lys	24.84	26.04	0.548	0.172
Arg	13.94[b]	15.07[a]	0.294	0.034

Values are represented by means±SEM. [a,b] Means in the same row with different superscript are significantly different at $P<0.05$. Control = Non-injected group. Arg = 1% L-Arginine group.

Table 4.7 Effects of in ovo feeding of Arg on amino acid compositions in breast muscle on D63 in Korat chickens (mg/g).

Items	Treatments		SEM	P value
	Control	Arginine		
Asp	21.78	22.11	0.536	0.701
Thr	10.42	10.24	0.286	0.663
Ser	8.91	9.01	0.277	0.811
Glu	36.78	37.13	0.754	0.754
Pro	10.46	11.27	0.497	0.293
Gly	14.92	14.27	0.45	0.344
Ala	17.78	17.19	0.492	0.428
Val	22.80	22.53	0.549	0.740
Met	6.69	6.91	0.189	0.486
Ile	17.41	17.55	0.473	0.838
Leu	25.15	24.95	0.358	0.754
Tyr	9.31	9.57	0.304	0.572
Phe	9.83	9.76	0.306	0.888
His	27.10	28.02	0.624	0.382
Lys	28.35	28.62	0.664	0.788
Arg	15.78	16.01	0.354	0.726

Values are represented by means±SEM. Control = Non-injected group. Arg = 1% L-Arginine group.

As presented in Table 4.7, amino acid compositions of breast muscle in D63 were not changed (P>0.05) by IOF of Arg compared to the control group.

4.1.5 Antioxidant capacity

The antioxidant capacity of breast muscle are shown in Table 4.8, MDA contents was lower (P<0.05) in Arg group when compared to the control group on DOH, but no significant difference were found (P>0.05) between two groups on D21, D42 and D63 respectively. There was significant difference (P<0.05) in the T-AOC activities of breast muscle on D21 between two groups, but no effect (P>0.05) on DOH, D42 and D63 respectively. GSH contents was higher (P<0.05) in breast muscle on DOH in Arg group compared to the control group, but no significant difference were observed (P>0.05) on D21, D42 and D63 respectively.

4.1.6 Chemical compositions

Compared to the control group, IOF of Arg significantly increased (P< 0.05) the dry matter and crude protein, but did not affect (P>0.05) the ash and ether extract in Korat chickens (Table 4.9).

4.1.7 Meat quality

As presented in Table 4.10, the meat quality (pH^{45min}, pH^{24h}, color, drip loss, cooking loss, shear force) were not significantly affected (P>0.05) by IOF of Arg compared to the control group.

In Table 4.11, no significant correlation was found (P>0.05) between meat quality and antioxidant capacity in Korat chickens.

Table 4.8 Effects of in ovo feeding of Arg on antioxidant capacity of breast muscle in Korat chickens.

Items	Treatments		SEM	P value
	Control	Arginine		
MDA (nmol/mg muscle)				
DOH	0.18[a]	0.11[b]	0.016	0.044
D21	0.35	0.28	0.032	0.195
D42	0.32	0.29	0.216	0.680
D63	0.38	0.34	0.027	0.345
T-AOC (nmol/mg protein)				
DOH	12.15	14.41	0.667	0.077
D21	3.67[b]	4.36[a]	0.114	0.011
D42	4.79	4.83	0.192	0.881
D63	4.16	4.33	0.168	0.492
GSH (nmol/mg muscle)				
DOH	4.58[b]	5.88[a]	0.181	0.012
D21	1.93	2.80	0.312	0.127
D42	1.84	2.13	0.199	0.387
D63	1.36	1.61	0.178	0.448

[a,b] Means in the same row with different superscript are significantly different at P< 0.05. Values are represented by means±SEM. MDA = Malondialdehyde; T-AOC = Total antioxidant capacity; GSH = Glutathione. Control =Non-injected group. Arg = 1% L-arginine group.

Table 4.9 Effects of in ovo feeding of Arg on chemical compositions of Korat chickens on D63 (%, DM basis).

	Treatments			
Items	**Control**	**Arginine**	**SEM**	**P value**
Dry matter (%)	26.19[b]	28.08[a]	0.796	0.024
Ash (%)	0.67	0.50	0.296	0.247
Ether extract (%)	1.57	1.36	0.169	0.413
Crude protein (%)	23.78[b]	25.52[a]	0.357	0.014

[a,b] Means in the same with different superscript are significantly different at P<0.05. Values are represented by means±SEM. Control = Non-injected group. Arg = 1% L-Arginine group.

Table 4.10 The effects of in ovo feeding of Arg on meat quality of Korat chickens on D63.

	Treatments			
Items	**Control**	**Arginine**	**SEM**	**P value**
PH^{45min}	5.99	5.91	0.082	0.640
pH^{24h}	5.84	5.83	0.062	0.849
Color				
L^*	52.24	51.81	1.274	0.819
a^*	2.33	2.23	0.297	0.832
b^*	1.95	1.26	0.3385	0.255
Drip loss (%)	12.06	10.49	1.050	0.351
Cooking loss (%)	22.94	24.68	1.137	0.462
Shear force (kg/cm^2)	1.99	2.28	0.092	0.125

Values are represented by means±SEM. Color: L^* = lightness; a^* = redness; b^* = yellow. Control = Non-injected group. Arg = 1% L-Arginine group.

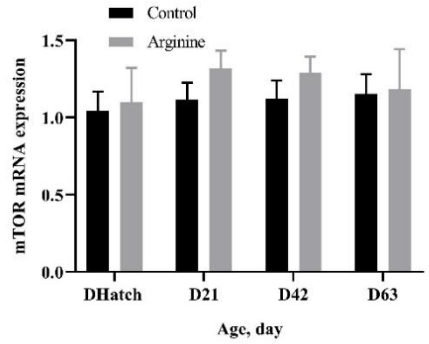
Table 4.11 Correlation coefficients between meat quality and antioxidant capacity in Korat chickens.

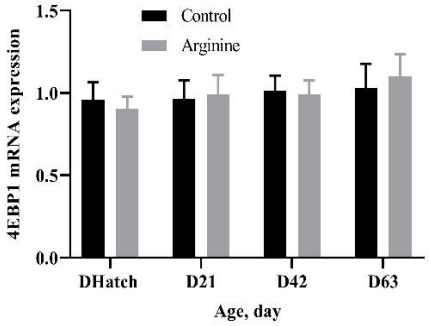
	pH^{45min}	**pH^{24h}**	**a***	**b***	**L***	**Drip loss**	**Cooking loss**	**Shear force**
MDA	0.682	0.479	-0.079	0.047	-0.372	-0.177	-0.796	-0.107
TAOC	0.099	0.166	-0.385	-0.342	0.690	0.341	0.690	0.087
GSH	-0.592	0.842	-0.865	-0.838	-0.017	-0.381	-0.108	-0.589

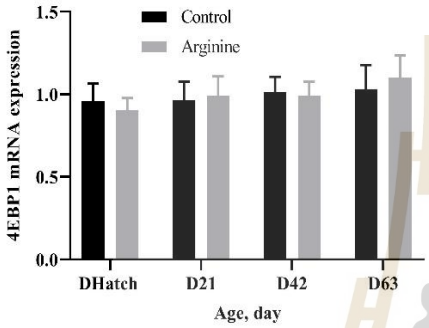
Color: L* = lightness; a* = redness; b* = yellow; MDA = Malondialdehyde; T-AOC = Total antioxidant capacity; GSH = Glutathione.

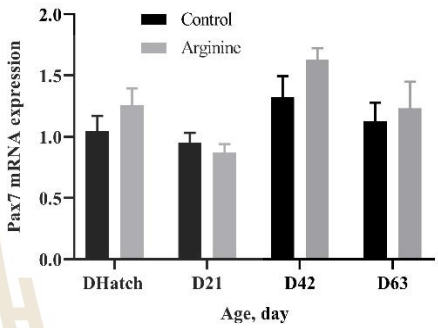
4.1.8 mRNA expressions related to the muscle development

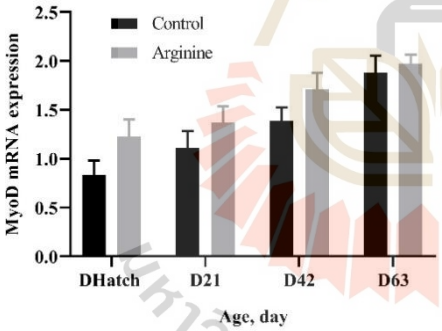
As indicated in Figure 4.2, The mRNA abundances of mTOR, 4EBP1 and S6K1 of mTOR signaling pathway in breast muscle were not affected (P>0.05) by IOF of Arg on DOH, D21, D42 and D63 post-hatch respectively. There was no significant differences (P>0.05) in Pax7 and MyoD mRNA abundances of myogenic genes between two groups on DOH, D21, D42 and D63 post-hatch respectively. The mRNA expression of Myf5 was found to be significantly up-regulated (P<0.05) in breast muscle of Arg group on D21 post-hatch compared with the control group. Compared to the control group, the mRNA expression of MyoG was greater (P<0.05) in breast muscle of Arg group on DOH and D21 post-hatch respectively, whereas MRF4 mRNA expression abundance of breast muscle was higher (P<0.05) in Arg group on DOH than that of control group.

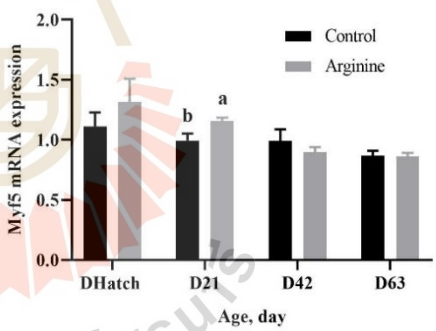


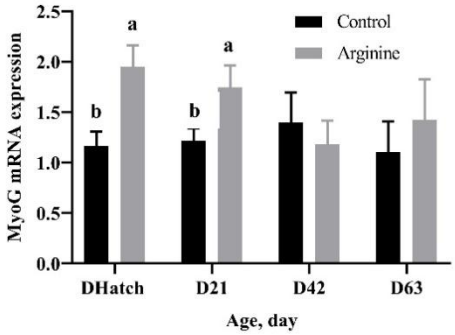












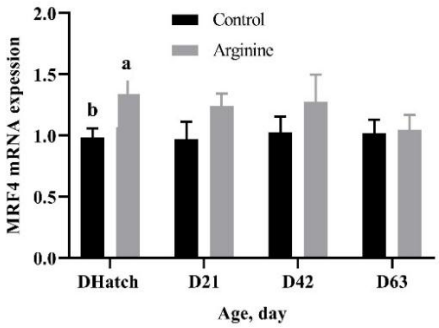


Figure 4.2 Effects of in ovo feeding of Arg on mRNA expressions related to the muscle development on DOH, D21, D42 and D63 post-hatch in Korat chickens. Values are represented by means±SEM. Different superscripts with the same time point indicate significant differences between two groups (P<0.05). Control =Non-injected group. Arg = 1% L-Arginine group.

4.1.9 Principal component analysis

PCA has ability to cluster the differcence between two groups based on breast muscle traits and genes expression of muscle development on DOH, D21, D42 and D63 respectively. PCA scoring and correlation loading plots are presented in 4 Figures. In Figure 4.3, PC1 explained 41% of variance and separated clustering of samples from the control and Arg group (Figure 4.3a), all parameters located outside the internal circle except for BMW and 4EBP1, indicating which positively correlaetd with Arg group (Figure 4.3b). In Figure 4.4, PC1 explained 40% of variance that distinguished all samples from the control and Arg group on D21 (Figure 4.4a). Most parameters (mTOR, 4EBP1, MRF4, Myf5, MyoD, and MyoG MFD, BMW) located outside the internal circle, indicating which mainly characterised in Arg group (Figure 4.4b). In Figure 4.5, PC2 accounted for 27% of variance that was not clear separation between control and Arg group on D42 (Figure 4.5a). mTOR, 4EBP1, Pax7, MyoD, MRF4 and BMW located outside the internal circle, indicating which higher characterised in Arg group (Figure 4.5b). In Figure 4.6, 27% of variance was explained in PC2, the clustering was not cleared separated by control and Arg groups, but showed a trend of separation on D63 (Figure 4.6a). mTOR, 4EBP1 , S6K1, Pax7, MyoD, MyoG, and BW located outside the internal circle, indicating which exhibited

a higher characters in Arg group (Figure 4.6b). Thus, PCA model is a good tool to distinguish the difference from Arg and control group and explained the relationship between breast muscle traits and genes expression of muscle development on DOH and D21 respectively. While the trend of grouping from two treatment were exhibited by PCA model on D42 and D63 respectively, However the relationship between breast muscle traits and genes expression of muscle development still can be found.

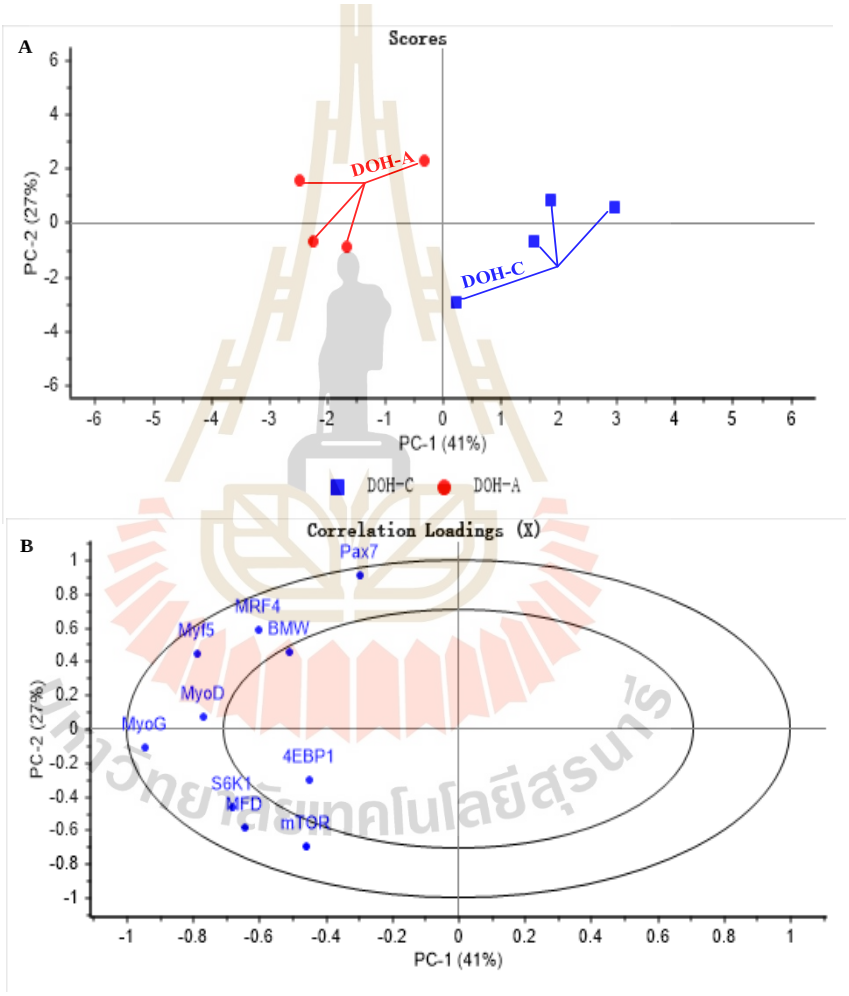


Figure 4.3 Score plot (A) and correlation loading plot (B) of principal component analysis (PCA) for gene expression and breast muscle traits on DOH. DOH-C = day of hatch-control group; DOH–A = day of hatch-Arg group; BMW = breast muscle weight, MFD = muscle fiber diameter.

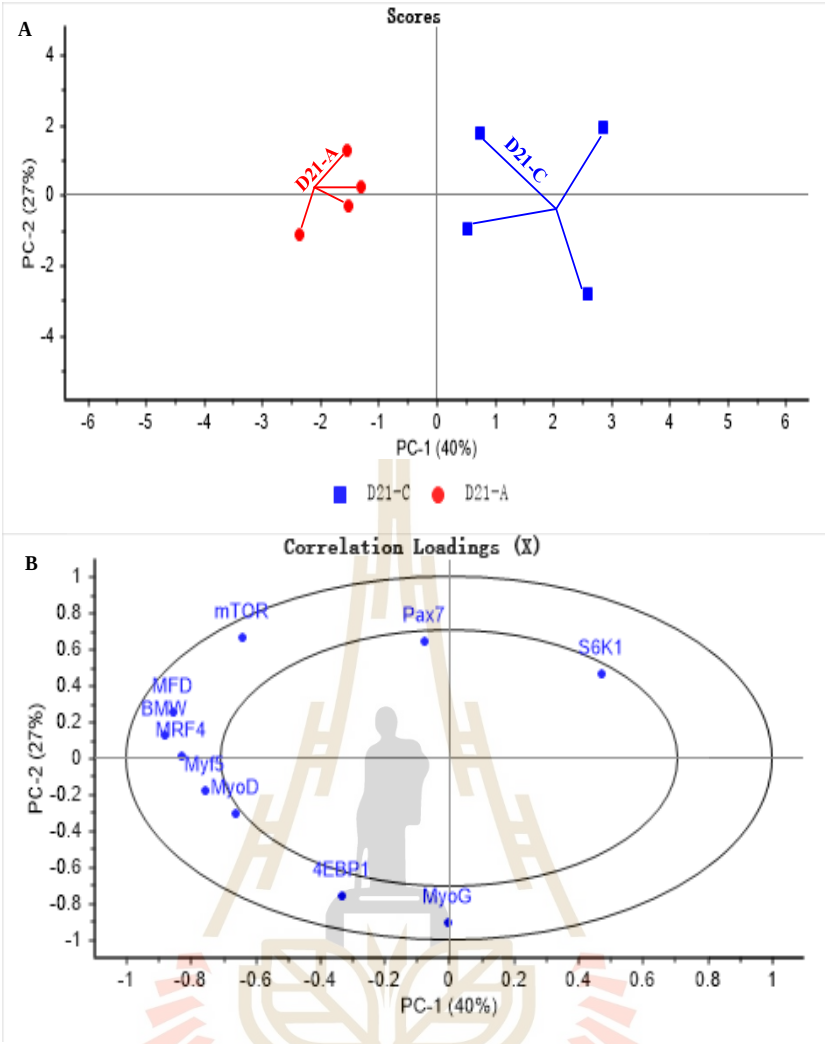


Figure 4.4 Score plot (A) and correlation loading plot (B) of principal component analysis (PCA) for gene expression and breast muscle traits on D21. D2-C = day 21- control group; D21-A = day 21-Arg group; BMW = breast muscle weight, MFD = muscle fiber diameter.

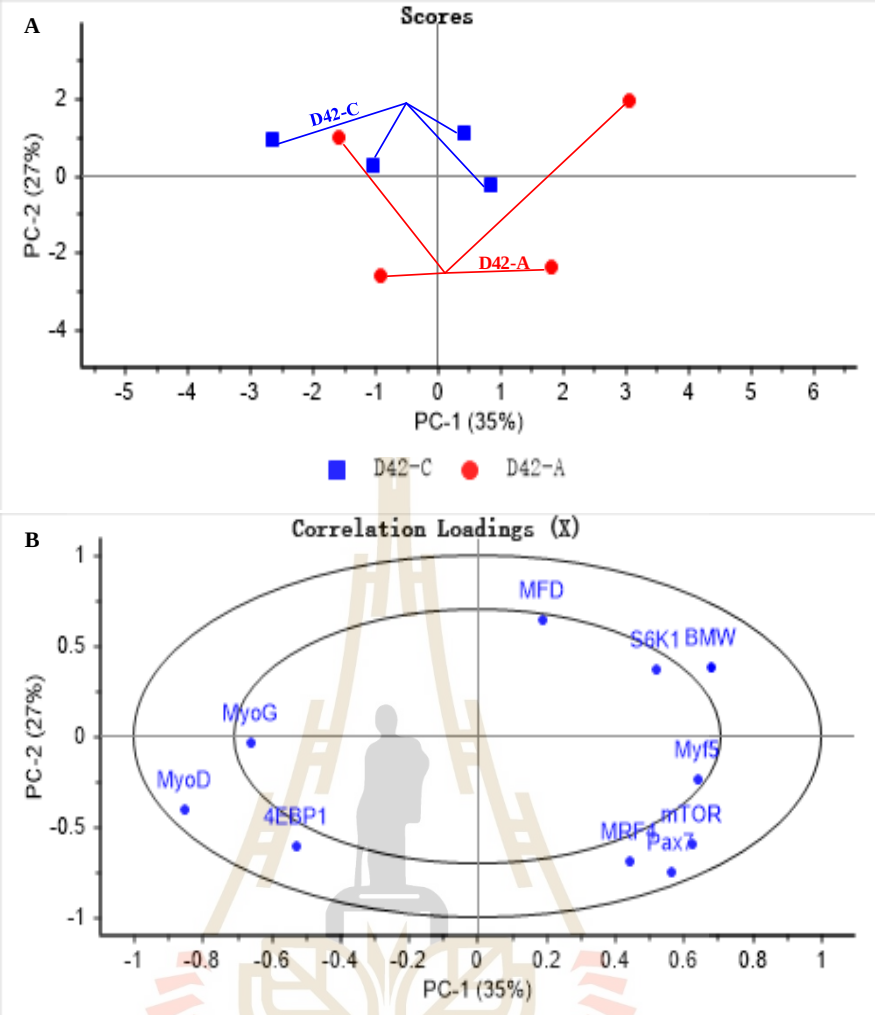


Figure 4.5 Score plot (A) and correlation loading plot (B) of principal component analysis (PCA) for gene expression and breast muscle traits on D42. D42-C = day 42- control group; D42-A = day 42-Arg group; BMW = breast muscle weight, MFD = muscle fiber diameter.

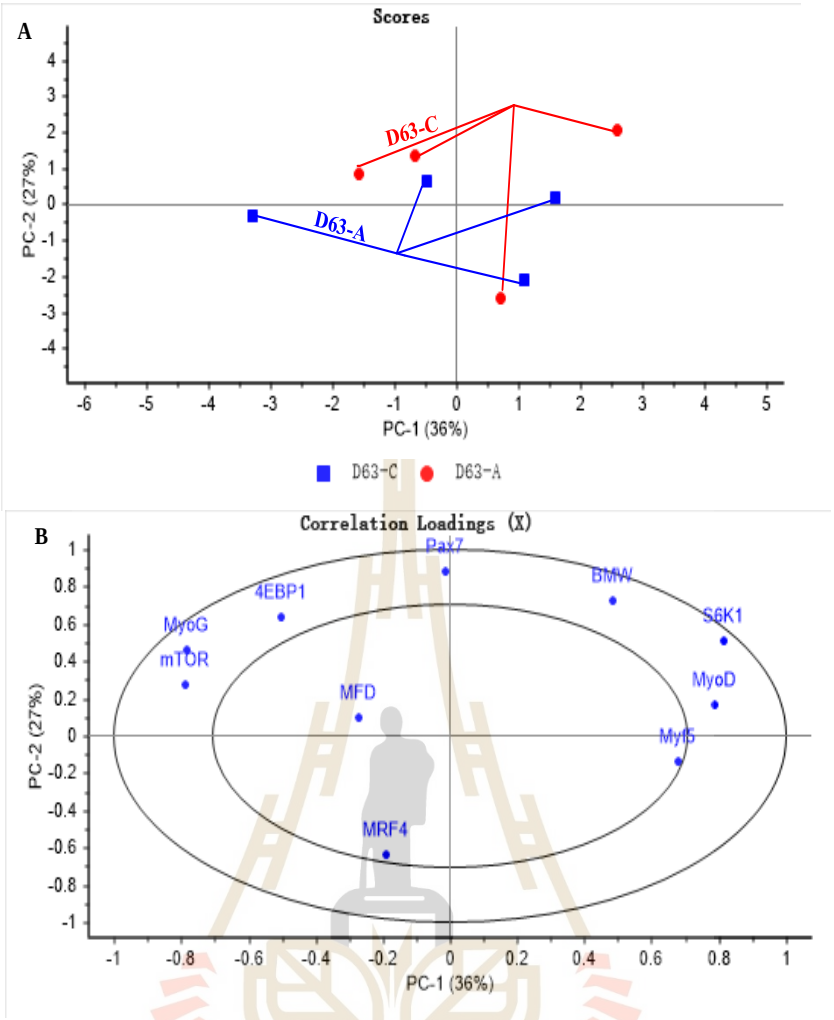


Figure 4.6 Score plot (A) and correlation loading plot (B) of principal component analysis (PCA) for gene expression and breast muscle traits on D63. D63-C = day 63-control group; D63-A = day 63-Arg group; BMW = breast muscle weight, MFD = muscle fiber diameter.

4.2 Discussion

4.2.1 Hatchability growth performance

In current study, the effects of IOF of Arg were studied on hatchability and growth performance (BW, BWG, FI, FCR) of Korat chickens in feeding period of 63 days. These undesired results were obtained that the hatchability, BW, BWG, FI

and FCR were unaffected by IOF of Arg in these 4 periods. Similarly, previous studies found different results from ours. Li et al. (2016) reported that IOF of Arg had no effect on BW in broiler chicken on D7, D14 and D21 post-hatch respectively. And, Fernandes et al. (2009) indicated that live weight was not affected by supplementing Arg from 1 to 21 days in broiler chickens. On the contrary, other studies found that the IOF of Arg had positive effects on poultry. Such as, Gao et al. (2018) demonstrated that IOF of Arg did not affect the hatchability and significantly increased the FI and BWG during 1 to 42 days post-hatch, reduced the FCR during 1 to 21 days post-hatch in broiler chickens. Toghyani et al. (2018) observed that IOF of Arg significantly increased the BWG and FI from 1 to 42 days post-hatch in broiler chickens. Zhang et al. (2017) reported that hatchability was increased and BW of pigeons were significant higher by IOF of Arg during DOH to D14 post-hatch. Also, Tangara et al. (2010) indicated that BW of ducks were increased on DOH, D3 and D7 post-hatch by IOF of Arg. Similar result was also stated that Japanese quails injected with Arg significantly affected the BW (Al-Daraji et al., 2012). In addition, dietary Arg increased BW, BWG, FI, or feed efficiency in broiler chickens (Deng et al., 2005; Khajali et al., 2011; Ebrahimi et al., 2014; Yu et al., 2018a). The differences in previous studies and our study may due to the different genetic background, Arg solution dose and embryo physical condition could impact on the effects of in ovo administration on chickens post-hatch. Whereas, to our unexpected results, suggesting that in ovo feeding technique is a safe way for embryo development. 1% Arg had no positive effect on their performance in Korat chickens. The possible reason is excess Arg in their body cause extra energy expenditure such as deamination and excretion, what is worse, unbalanced amount of one amino acid leads to the toxic reaction (Benevenga and Steele, 1984).

4.2.2 Carcass traits

In the present study, IOF of Arg had no effect on carcass traits, including dressing yield, eviscerated yield, abdominal fat, heart, liver and gizzard in Korat chickens. In addition, the breast muscle weight were no significant difference from DOH-D63 post-hatch between two groups. Similarly, Li et al. (2016) reported that IOF of Arg had no or even negative effect on the pectoralis major weight in broiler chickens on D7, D14 and D21 post-hatch respectively. Fernandes et al. (2009) stated that dietary Arg did not affect the carcass weight, breast muscle yield of broiler chickens in the starter period. And, Khajali et al. (2011) also reported that dietary Arg did not affect carcass yeild, liver, breast and thigh meat yield. Conversely, Yu et al. (2018b) found that IOF of 1% Arg increased the relative breast muscle weight of broiler chickens on DOH, D7 and D21 post-hatch respectively. Gao et al. (2017) demonstrated that IOF of 1% Arg increased organs weight in broiler chickens on D7 and D21 post-hatch respectively. Still, according to the report of Zhang et al. (2017), who demonstrated that carcass weight, breast muscle weight and organs of pigeons were significantly increased on D7 and D14 post-hatch by IOF of 1% Arg. Moreover, Wu et al. (2011) found that breast muscle weight of ducks was increased by supplementing Arg in the diet. From the above previous studies, we knew that IOF of Arg into hatching eggs that must meet the requirements of optimal concentration or volume, which can positively affected the physiological traits and performances of chickens (Berrocoso et al., 2017). It also should be suggested that environmental factors is considered as the first consideration, because the embryo development of avians is separated from their female body, and the incubation temperature is the most important factor that interfere with embryonic development (Maltby et al., 2004), slight changes of temperature in incubation process affect embryonic development and

chicken development post-hatch (Liu et al., 2015; Maatjens et al., 2016). From the current obtained results, suggesting that the further detailed experiment should be do for confirming optimal dose of Arg solution in order to achieve the maximum growth of Korat chickens.

4.2.3 Muscle fiber traits

Muscle fiber number and muscle fiber diameter decide the muscle development. Muscle fiber number is fixed at hatch and muscle fiber diameter was achieved after-hatch. In this study, we checked the muscle fiber number on market day and muscle fiber diameter from DOH to D63 that follow our objective. The muscle fiber number was not increased on the market day by IOF of Arg. After hatching, the muscle develop rapidly by the increase of muscle cell size that accumulating the satellite cells. Accroding our results, muscle fiber diameter were not differences between two groups from DOH to D63. In addition, we also found that the muscle fiber diameter enlarged much during starter period (DOH-D21) than grower and finisher periods (D21- D63), it indicates that the muscle weight or BW growth fastly in starter stage, sufficient early nutrient supply is necessary for young poultry (Zhao et al., 2017; Xu et al., 2020). According to the report of Kornasio et al. (2011), who demonstrated that IOF procedure on D18 of incubation showed positive effect on muscle cell numbers in embryo, as well as the muscle fiber diameter was larger on D35 post-hatch and played a long-term effect on breast muscle growth. Previous study confirmed that delayed muscle fiber maturation attribute to the late-feeding and irreversibly impared muscle develoment (Halevy et al., 2000). Moreover, dietary supplementation with Arg positively responed to the muscle fiber diameter in broiler chickens (Fernandes et al., 2009). The explanation of this resullts will be found by the detailed mechanism that are explored and discussed in the following sections.

4.2.4 Amino acid compositions

Amino acid compositions of embryo, yolk, albumen, allantoic and amnion fluids were affected by IOF of amino acid in broiler chickens (Ohta et al., 2001). So, in ovo technique may be an effective way to improve the amino acid compositions in order to improve the amino acid utilization. In the present study, we estimated the amino acid compositions of breast muscle on DOH, D21, D42 and D63 post-hatch by IOF of Arg. Taken together, these results showed most of amino acids were affected in breast muscle on DOH. On D21, Arg, Pro and Phe were affected. On D42, Val, Ile, His and Arg were affected respectively, but no effect on D63. These finding were consistent with previous studies, Yu et al. (2018b), reported that IOF of Arg affected the most of amino acids in breast muscle on DOH, D7 and D21 post-hatch respectively in broiler chickens and increased breast muscle weight. Gao et al. (2017) demonstrated that IOF of Arg increased the concentrations of Val, Met, Ile, Leu, Arg and Pro in serum of broiler chickens and improved body growth on D21 post-hatch. Remarkably, dietary Arg increased the Arg concentration of plasma but not affected the body weight gain in broiler chickens on D15 (Kidd et al., 2001). For our results, indicating that supplemental Arg into embryo that is able to modulate amino acid compositions and accumulate them in breast muscle until D42 post-hatch in Korat chickens. IOF of amino acid in egg improve amino acid accumulation and change their composition that may stimulate amino acid utilization through increasing amino acids synthesis and decreasing amino acids degradation for maximum growth (Ohta et al., 2001). Our results also indicates that the change of amino acid compositions in Arg group cannot respond to the muscle mass on different ages post-hatch. The explanation may be the amino acid utilization cannot be improved by supplemental Arg into embryo. As the report of Al‐Murrani (1982), noting that

supplementing amino acid to the embryo that must be the same as original amino acid pattern of egg that resulted in the increasse of BW post-hatch in broilers.

In addition, Arg is substrate of Pro that is produced via arginase. Pro is involved in collagen synthesis of muscle (Listrat et al., 2016), it is proposed that increased Pro may be benefit to the muscle development. Creatine is related to the energy metabolism in muscle and brain (Curt et al., 2015; Yang et al., 2019). Nitric oxide, which has function in immune and cardiovascular system (Wu et al., 2009). Speculating that Arg may go to these way to act the metabolic activity that are benefit for chicken health.

4.2.5 Antioxidant capacity

Oxidative stress is the end process of stressors, is generated from reactive oxygen species (ROS), which is harmful for animal health, and may cause the cellularity damage, animal diseases and the decrease of production efficiency result in lipid peroxidation, protein oxidation and DNA modification (Zabłocka and Janusz, 2008; Xiao et al., 2016). Malondialdehyde, is known as a biomarker to monitor the degree of oxidative stress. In the present study, we found that IOF of Arg decreased MDA content on DOH. This result was in agreement with those of previous studies. Such as, Duan et al. (2015) found that supplementing Arg in late laying hen's diet significantly reduced the MDA contents in serum, egg yolk and serum of broiler breeders as well as the liver and breast muscle of broliers on D1 post-hatch. Atakisi et al. (2009) reported that dietary Arg to Japanase quails decresed the MDA content in blood. Similarly, the decrease of MDA contents were found by supplementing Arg in piglets and rats (Xiao et al., 2016; Zheng et al., 2018). In the present finding, indicating that in ovo administation of Arg can improve antioxidant status though anti-lipid peroxidation in Korat chickens.

Antioxidant balance plays important role for normal physiological metabolic activity in animals. In our study, antioxidant capacity of GSH and T-AOC were determined. GSH can decrease the oxidative stress of cells, due to it is related to enzymatic processes, that can reduce H_2O_2 by GSH metabolism into oxidized glutathione and other hybrid disulfides. So, which is biomarker of cellular antioxidant defense capacity (Hung et al., 2006). Xiao et al. (2016) reported that adding Arg in rat's diet, increasing the GSH level of jejunum. In the present study, GSH level was increased on DOH by IOF of Arg. This result indicates that Arg has ability to improve the antioxidant capacity of breast muscle. T-AOC, is used as integrative indicator of total antioxidant capacity in animal body (Ren et al., 2012). According the report of Duan et al. (2015), who indicated that dietary supplementation with Arg increased T-AOC activities in serum and egg yolk of laying hen as well as serum, liver and breast of broilers on D1 post-hatch. Atakisi et al. (2009) reported that dietary Arg to Japanese quails improved T-AOC activity. In addition, Arg displayed the positive results for T-AOC activities to pigs and rats (Ma et al., 2010; Xiao et al., 2016). In our study, T-AOC activity was significantly increased by IOF of Arg on D21. This result suggests that IOF of Arg enhanced the T-AOC antioxidant capacity of breast muslce.

Based on foregoing founding, Arg has positive role against lipid peroxidant and enhance the antioxidant capacitiy of breast muscle. It can keep antioxidant function during starter period, which is crucial stage for body growth and may improve production of Korat chickens.

4.2.6 Chemical compositions

The results of this study verified that IOF of Arg affected the dry matter and crude protein in Korat chickens, This study was consistent with previous report (Ebrahimi et al., 2014), who found that Arg in the diet increased the dry matter

and crude protein in broiler chickens. Wu et al. (2011) and Sathiyapriya et al. (2018) also found that Arg supplementation positively affected on the crude protein in ducks. However, in this study, there was contradiction between crude protein and amino acids. Crude protein contains true protein and non-protien nitrogen, true protein is made up of amino acids. So, the probable reason may be the IOF of Arg caused more non-protein nitrogen was contained in crude protein rather than true protein.

4.2.7 Meat quality

Meat quality is closely associated with purchasing desire of consumers. pH value should be concerned firstly. In this study, pH value at 45 min and 24 h post-mortem were similar between control and Arg group, these values observed were within the accept range for chickens (Zhang and Barbut, 2005). In general, color is considered the important indicator of meat quality, the pH of meat was highly related to color (Fletcher et al., 2000). The meat colors in this study were not affected by IOF of Arg. These results were consistent with the previous studies, the authors indicated that dietary supplementation with Arg (0.5% or 1%) did not affect the pH value (at 45 min, 24 or 48 h post mortem) and color in pigs (Ma et al., 2010; Hu et al., 2017; Shi et al., 2019). On the contrary, Shi et al. (2018) demonstrated that dietary 1% Arg supplementation in sows had lower b^* value, but did not affect pH value in their progeny.

Regarding the drip loss, cooking loss and shear force, which are also the important indicators of meat quality for detecting sensory characteristics (tenderness, juiciness and flavor) (Aaslyng et al., 2007). Shi et al. (2018) reported that supplementing 1% Arg in sows did not affect the drip loss, cooking loss and shear force in their progeny. Similarly, Hu et al. (2017) demonstrated that drip loss and cooking loss in growing-finishing pigs were not affected by adding 1% Arg in diet.

Moreover, through the reports of Oksbjerg et al. (2019), who suggested that dietary supplementation with 25 g/d of Arg in sows had no improvement in cooking loss or shear force of meat in their offspring. As to the current study, IOF of Arg did not affect any parameters of meat quality in Korat chickens, it was proved that in ovo administration had no positive or negtive effect on meat quality. In addition, the antioxidant capacity is related to the the meat quality. In our study, we did not find any correlation between them. Due to there is limited information about the effects of Arg (dietary or in ovo feeding) on the chicken meat, the possible reason of this study is the long internal between in ovo feeding to market age.

4.2.8 mRNA expression related to the muscle development

Arg is a one of nutrition factors that can improve the muscle mass development via the protein deposition dependent on mTOR signaling pathway under their two downstream target proteins of S6K1 and 4EBP1 in skeletal muscle. However, in our study, the mRNA expressions of mTOR, 4EBP1 and S6K1 of breast muscle did not differ by IOF of Arg in Korat chickens. This results were inconsistent with previous studies, for example, Yu et al. (2018b) demonstrated that IOF of Arg into breast muscle of broiler chickens increased mRNA expressions of mTOR, 4EBP1 and S6K1 and breast muscle weight during growing period. Gao et al. (2018) demonstrated that IOF of Arg positively regulated intestine development through stimulating mTOR signaling pathway. In addition, Yuan et al. (2015) also proved that the addition of Arg in broiler chickens diet increased the mRNA expressions of mTOR, 4EBP1 and S6K1 in intestinal epithelial cells and enhanced the protein synthesis in vitro experiment. Inspiringly, our results was in line with report by Wei et al. (2021) in fish species. To our results, suggesting that the gene expression involved in mTOR signaling pathway may has the different patterns in different strains of

poultry. Because Arg also has ability to secrete growth hormone (GH), IGF-1 and insulin (Stechmiller et al., 2005), which can activate the mTOR signal transduction pathway then stimulate protein synthesis (Bolster et al., 2004; Tu et al., 2015). Davis et al. (2002) reported that protein synthesis was stimulated by insulin and amino acids in skeletal muscle of neonatal pigs. Xu et al. (2018) revealed that dietary Arg enhances the secretion of GH, IGF-1 and insulin then increased the growth performance in broiler chickens. It is possible that Arg and GH/IGF-1/insulin have a joint effect on protein synthesis in slow growing chickens. Thus, we speculates from the current result that mTOR signaling pathway is controlled by Arg and insulin /GH/IGF-1 in Korat chickens.

The nutritional regiment can modify the genes expression of myogenesis from activating satellite cells to fuse with muscle fiber that offer great potential for muscle hypertrophy. Satellite cell is specialized as myogenic precursor cell. In post-natal phase, the proliferation and differentiation of satellite cells are controlled by the myogenic regulatory factors, including MyoD, Myf5, MyoG and MRF4. Specifically, MyoD and Myf5 are known as the early or commitment myogenic regulatory factors in the MRFs family of transcription factors (Francetic and Li, 2011). In addition, Pax7 is an important regulator of satellite cells and plays an important role in maintain a population of quiescent satellite cells (Knight and Kothary, 2011). In our study, expectedly, Myf5 mRNA expression was up-regulated on D21 post-hatch by IOF of Arg. Genes expression of Pax7 and MyoD did not differ in their all life. The present data were consistent with report of Li et al. (2016), who also found that the mRNA expressions of Pax7 and MyoD did not affected by culturing muscle cells of broiler chickens by IOF of Arg. Implying that myoblast proliferation of breast muscle was achieved by Myf5 gene, which plays important role

in proliferation and maintenance of myogenic-lineage specification and are showed in undifferentiated proliferating myoblasts and myotubes (Braun et al., 1989). However, Kablar et al. (1997), who indicated that Myf5 expression in the limb is insufficient for the myogenic development. Both of them are necessary to regulate the proliferation of myoblasts from myogenic precursor cells for acquisition of the myoblast phenotype (Cossu et al., 1996).

The other two members of MFRs family are MyoG and MRF4. MyoG is expressed in the early steps of terminal differentiation (Nabeshima et al., 1993). In the current study, MyoG mRNA expression of breast muscle was up-regulated on DOH and D21 post-hatch respectively. This result was consistent with Subramaniyan et al. (2019), who demonstrated that MyoG protein was up-regulated during embryogenesis by IOF of Arg. MRF4 contributes to the later steps of myotube maturation (Zhang et al., 1995). Our data showed that MRF4 mRNA expression was increased on DOH by IOF of Arg. Previous study has been proved that MyoG induction is indispensable and sufficient for the formation process from myotubes and to fibers (Knapp et al., 2006).

Suggesting that both of MyoG and MRF4 promote the terminal differentiation of myoblasts into myocyte, fusing together to form a mature multinucleate myofiber in starter period. Moreover, it is speculated that the two genes have no main responsibility for the enlargement of muscle fiber diameter, due to the satellite cells numbers are limited. As the report of Gayraud-Morel et al. (2007) indicated that the MRF4 and MyoG of differentiation factors are not participated in satellite cells development or maintenance. Interestingly, the data of Pax7 and MyoD genes in our study were strong evidence that satellite cells were not activated and

proliferated that may limited myogenic specification of satellite cells, finally influencing on the muscle fiber hypertrophy.

4.2.9 Principal component analysis

Satellite cells activity alters with age that affect the genes expression of myogenesis at the different age. Muscle deveopment invovled in Pax7, MyoD, Myf5, MyoG, MRF4, mTOR, 4EBP1 and S6k1, that are responsible for muscle hypertrophy and muscle weight. The results of PCA models showed the significant correlation of genes in muscle development, indicating that genes of MRFs and mTOR signaling pathway work together to help the formation of muslce fiber to affect the breast muscle weight after IOF of Arg. Aguiar et al. (2013) confirmed that the MRFs genes were positively correlated with muscle hypertrophy in rats. Rion et al. (2019) also reported mTOR signaling pathway was responsible for controlling the myogenic process. In our results, the genes of mTOR, S6K1, Pax7 and MRFs family related to the MFD on DOH. On D21, the genes of mTOR, 4EBP1 and MRFs familty respond to the MFD and BMW. Indicating that supplemental Arg to embryo had obvious role on muscle development on DOH and D21 respectively. However, the effect of IOF of Arg on muscle development is diminishing with age. On D42, mTOR, 4EBP1, Pax7, MyoD and MRF4 associated with BMW, while mTOR, 4EBP1, S6K1 Pax7, MyoD and MyoG were correlated to the BMW on D63. These results imply, even though the effects of Arg on muscle development is to diminish on the last two periods than the first two periods, which still has ability to support the muscle development. mTOR gene knockout reduced myogenic genes expression (Zhang et al., 2015). Speculating that mTOR signaling pathway promote the MRFs expression, the interaction of these genes may response to the muscle development by IOF of Arg to some extent. Which has a potential positive role in muscle development.

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CHAPTER V

CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

IOF of 1% Arg did not improve the growth performance, carcass traits, muscle fiber traits and mRNA expressions of mTOR, S6K1, 4EBP1, Pax7 and MyoD during feeding period of 63 days, and did not affect the meat quality. However, amino acid compositions can be changed from DOH to D42. Arg plays an important role in antioxidant capacity and genes expression related to the muscle development during starter period. Specifically, IOF of Arg decreased MDA contents, increased antioxidant levels of GSH and T-AOC, mRNA expressions of Myf5, MyoG and MRF4 were increased during DOH to D21. This may be the gene expression pattern of myogenesis in breast muscle of Korat chickens after IOF of Arg. In addition, muscle growth traits and genes of muscle development were analyzed by principal component analysis (PCA) and showed that there is clear separation between control and Arg groups on DOH and D21 respectively, while a trend of grouping from two groups were exhibited on D42 and D63 respectively.

Thus, IOF of 1% Arg may contribute to the muscle differentiation to form myotube rather than proliferation. Which may have potential role to improve muscle development in Korat chickens.

5.2 Implication and recommendation

"Korat chicken" has high meat quality with the less muscle. In order to meet the demand of market that the every parts of chicken body can be segmented for selling. So, this study was conducted that in ovo technique will be a new way to help the muscle development with maintaining high meat quality. Which is benefit to Korat chicken's production.

From this study, we found that in ovo technique is benefit for the chicken growth and has no negative effect on the meat quality. However, IOF of 1% Arg did not increase the breast muscle weight and body weight, suggesting that further study is needed that choose the optimal dose (less than 1% or 0.5 ml) of Arg to inject to the embryo. The expected outcome might be found in the future work.

APPENDIX

Figure A1 Electrophoresis of total RNA.

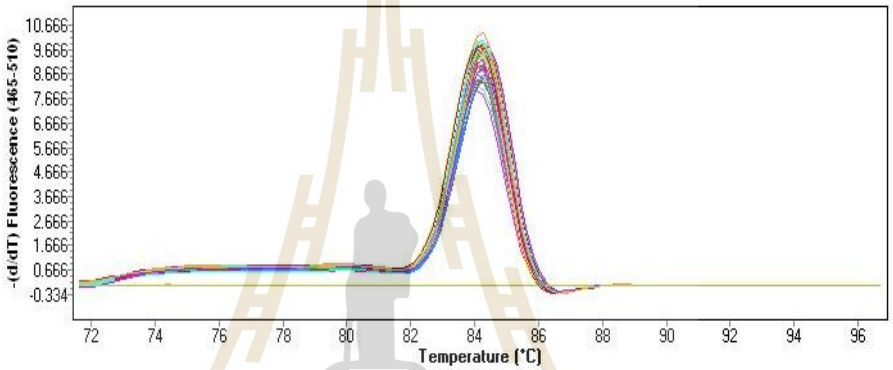

Figure A2 Melting curve of GAPDH gene.

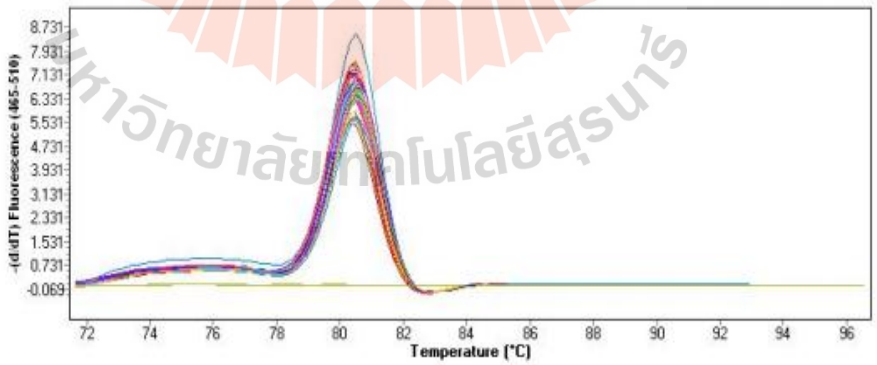

Figure A3 Melting curve of mTOR gene.

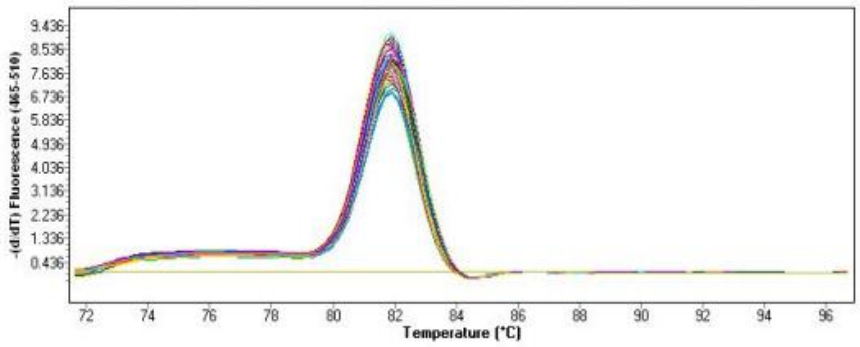

Figure A4 Melting curve of 4EBP1 gene.

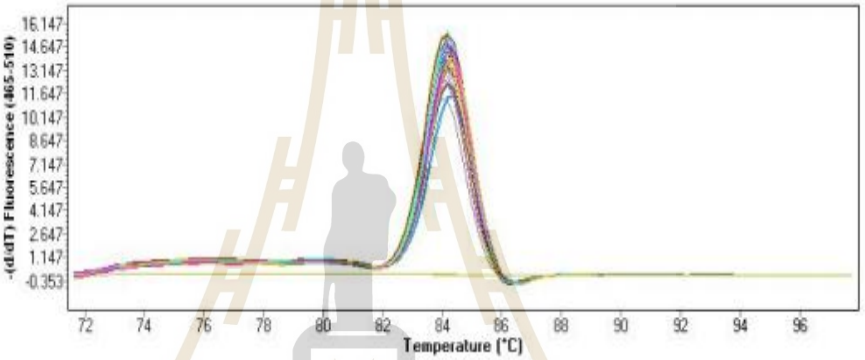

Figure A5 Melting curve of S6K1 gene.

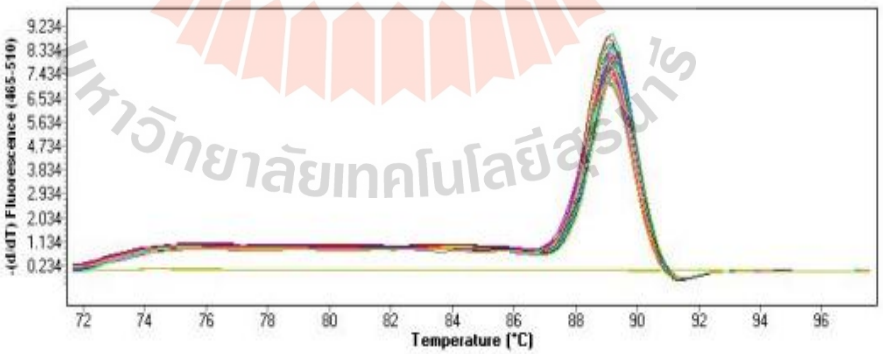

Figure A6 Melting curve of MyoG gene.

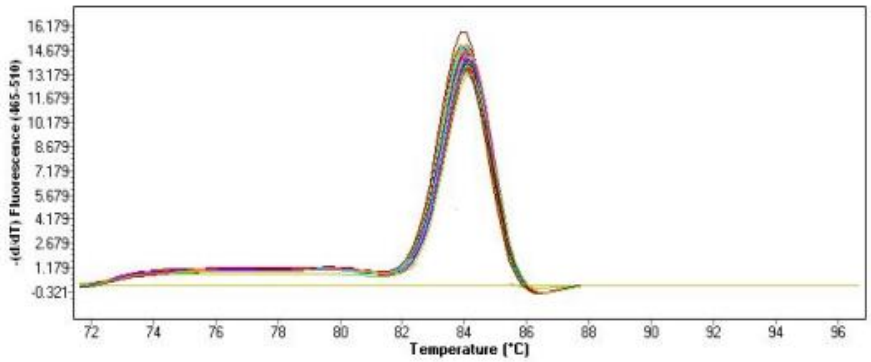

Figure A7 Melting curve of Pax7 gene.

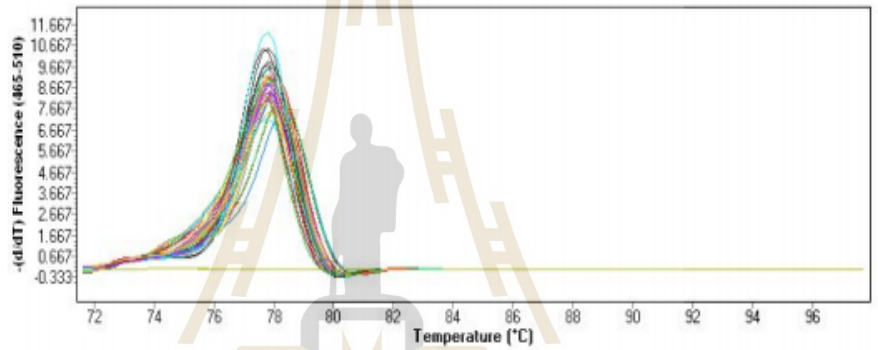

Figure A8 Melting curve of MyoD gene.

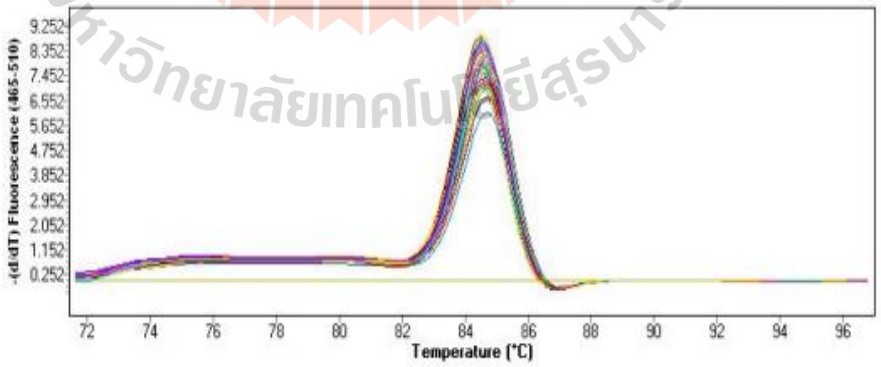

Figure A9 Melting curve of Myf5 gene.

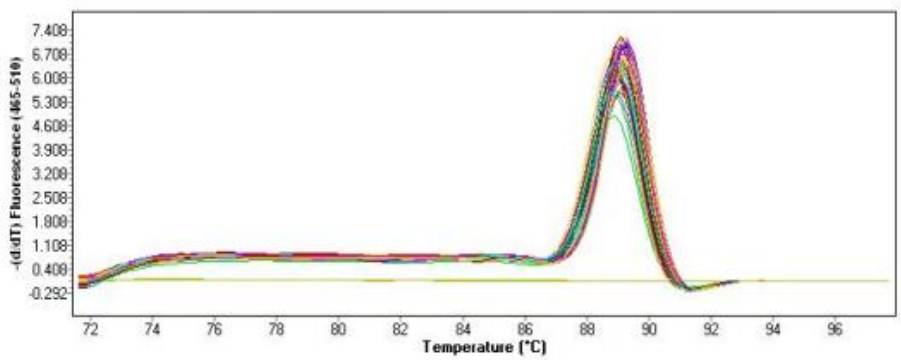

Figure A10 Melting curve of MRF4 gene.

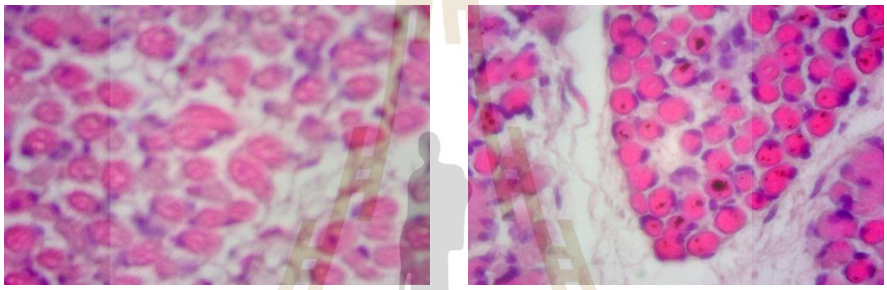

Figure A11 Observations of the breast muscle fiber sections on hatch day (left side = control group; right side = Arg group).

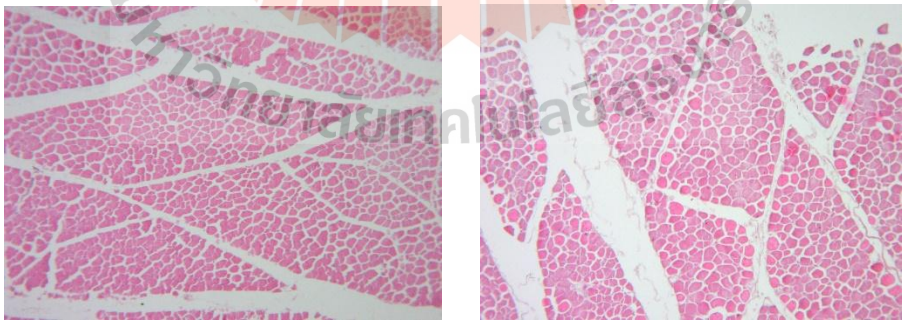

Figure A12 Observations of the breast muscle fiber sections on 21 day post-hatch (left side = control group; right side = Arg group).

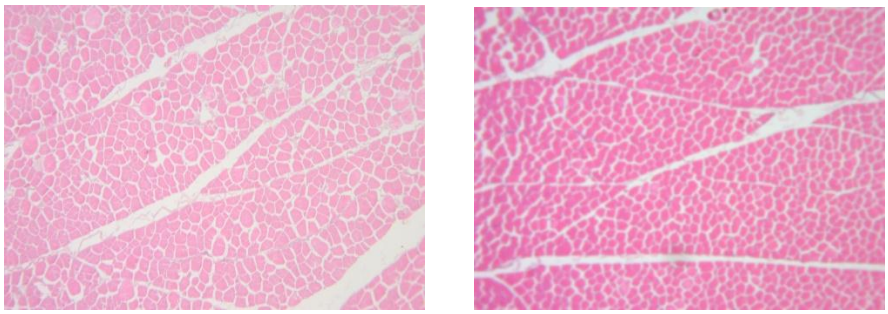

Figure A13 Observations of the breast muscle fiber sections on 42 day post-hatch (left side = control group; right side = Arg group).

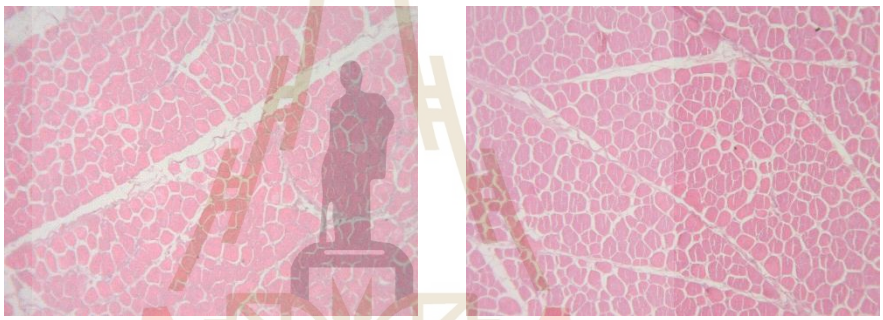

Figure A14 Observations of the breast muscle fiber sections on 63 day post-hatch (left side = control group; right side = Arg group).

Figure A15 The process of in ovo feeding of Arg.

Figure A16 The incubation of embryonic eggs.

BIOGRAPHY

Miss Panpan Lu was born in 12th June, 1987 in Anyang, Henan province, P. R. China. She graduated in 2011 from Henan University of Science and Technology with Bachelor degree in animal science, and, got Master degree of animal nutrition and feed science from Guizhou University in 2015. Then, she received scholarship of PhD program from Suranaree University and Technology (SUT), Thailand. She studied in the field of animal nutrition in School of Animal Technology and Innovation, Institute of Agricultural Technology. Her research direction of thesis were poultry nutrition, muscle development and meat quality.