# IDENTIFICATION OF GENES INVOLVED IN FEED EFFICIENCY AND MEAT QUALITY OF KORAT CHICKEN

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

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# การค้นหากลุ่มยีนที่มีบทบาทต่อประสิทธิภาพการใช้อาหารและคุณภาพเนื้อในไก่โคราช

นางสาวโชติมา ภูมิประหมัน



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาปรัชญาดุษฎีบัณฑิต
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# IDENTIFICATION OF GENES INVOLVED IN FEED EFFICIENCY AND MEAT QUALITY OF KORAT CHICKEN

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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โชติมา ภูมิประหมัน : การค้นหากลุ่มยีนที่มีบทบาทต่อประสิทธิภาพการใช้อาหารและคุณภาพเนื้อในไก่โคราช (IDENTIFICATION OF GENES INVOLVED IN FEED EFFICIENCY AND MEAT QUALITY OF KORAT CHICKEN) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร.อมรรัตน์ โมพี, 115 หน้า.

การศึกษานี้มีวัตถุประสงค์ข้อที่หนึ่งเพื่อศึกษาความสัมพันธ์ระหว่างประสิทธิภาพการใช้อาหาร (feed conversion ratio ; FCR, residual feed intake ; RFI) และคุณภาพเนื้อในไก่โคราช โดยใช้ไก่โคราชเพศผู้จำนวน 75 ตัว ทำการแยกเพศไก่ที่อายุ 1 วัน เก็บข้อมูลน้ำหนัก และปริมาณการกินได้แบบรายตัวตั้งแต่สัปดาห์ที่ 1-10 เก็บข้อมูลน้ำหนัก และการกินได้รายตัวรายสัปดาห์ เพื่อคำนวณค่า FCR กับ ค่า RFI เชือดที่อายุ 70 วัน ตัวอย่างเนื้ออก และเนื้อสะโพกวัดค่า ultimate pH, ค่า drip loss และ ค่า water holding capacity (WHC) ผลการศึกษาพบว่า ในกรณีเนื้ออกพบความสัมพันธ์แบบลบระหว่างค่า FCR ที่อายุ 10 สัปดาห์ กับค่า ultimate pH และ ค่า drip loss ในขณะที่พบความสัมพันธ์แบบบวกระหว่างค่า FCR ที่อายุ 10 สัปดาห์ กับค่า WHC ในกรณีเนื้อสะโพกพบความสัมพันธ์แบบลบระหว่างค่า FCR ที่อายุ 10 สัปดาห์ กับค่า drip loss การศึกษานี้ไม่พบความสัมพันธ์ระหว่างค่า RFI ที่อายุ 10 สัปดาห์ กับ ค่า ultimate pH, ค่า drip loss และค่า WHC ในเนื้ออก และเนื้อสะโพก นอกจากนี้ศึกษาความสัมพันธ์ระหว่างประสิทธิภาพการใช้อาหาร (FCR, RFI) กับสารตั้งต้นของรสชาติ (nucleotides content) และองค์ประกอบทางเคมีในเนื้อสะโพก โดยใช้วิธี High Performance Liquid Chromatography (HPLC) และ Fourier Transform Infrared (FTIR) spectroscopy ตามลำดับ ผลการศึกษาพบว่า ค่า FCR ที่อายุ 10 สัปดาห์ มีความสัมพันธ์แบบลบกับค่า Adenosine monophosphate (AMP) กับ inosine ในขณะที่ค่า RFI ที่อายุ 10 สัปดาห์ มีความสัมพันธ์แบบบวกกับค่า Inosine monophosphate (IMP) จากผลการศึกษาชี้ให้เห็นว่า การปรับปรุง FCR ส่งผลต่อความสามารถในการอุ้มน้ำในเนื้ออก และเนื้อสะโพก และพบว่าค่า FCR กับ RFI ส่งผลต่อรสชาติในเนื้อสะโพก

การศึกษานี้มีวัตถุประสงค์ข้อที่สองเพื่อค้นหากลไก และกลุ่มยีนที่เกี่ยวข้องกับประสิทธิภาพการใช้อาหาร และคุณภาพเนื้อในเนื้อสะโพกไก่โคราช โดยสกัด total RNA จำนวน 21 ตัวอย่าง จากเนื้อสะโพกไก่กลุ่มที่มีค่า FCR สูง กับ ไก่กลุ่มที่มีค่า FCR ต่ำ ใช้เทคนิค microarray เพื่อสืบหาข้อมูลการแสดงออกของยีน ใช้วิธี Weighted gene coexpression network analysis (WGCNA) ในการค้นหากลไก และกลุ่มยีนที่เกี่ยวข้องกับประสิทธิภาพการใช้อาหาร และคุณภาพเนื้อ ผลการศึกษาพบว่ากลไก mitochondrial gene expression, mitochondria respiratory chain complex assembly, mitochondrial translation and positive regulation of mitochondrial translation และยีน

ACD, ยีน BIRC5, ยีน COA3, ยีน MYL9 มีความสัมพันธ์กับลักษณะประสิทธิภาพการใช้อาหารกับคุณภาพเนื้อในเนื้อสะโพกไก่ที่เจริญเติบโตช้า



สาขาวิชาเทคโนโลยีและนวัตกรรมทางสัตว์ ลายมือชื่อนักศึกษา_____

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ลายมือชื่ออาจารย์ที่ปรึกษาร่วม_____

CHOTIMA POOMPRAMUN : IDENTIFICATION OF GENES INVOLVED IN FEED EFFICIENCY AND MEAT QUALITY OF KORAT CHICKEN. THESIS ADVISOR : ASSOC. PROF. AMONRAT MOLEE, Ph.D., 115 PP.

FEED EFFICIENCY/MEAT QUALITY/TRANSCRIPTOMIC ANALYSIS/KORAT CHICKEN

This study had two aims. The first study aim was to investigate the correlations between feed efficiency (feed conversion ratio; FCR, residual feed intake; RFI) and meat quality in Korat (KR) chicken using 75 male KR chickens that were sexed at 1 day of age. Individual body weight and feed intake were recorded weekly for the calculation of the Feed Conversion Ratio (FCR) and the Residual Feed Intake (RFI). At 10 weeks of age, the birds were slaughtered, the breast and thigh were collected to measure the ultimate pH, drip loss and water holding capacity (WHC). Regarding the breast, FCR at 10 weeks of age was negatively correlated with ultimate pH and drip loss while positively correlated with WHC. FCR at 10 weeks of age was negatively correlated with drip loss. RFI was not correlated with ultimate pH, drip loss and WHC in breast and thigh. Moreover, the correlations between feed efficiency (FCR, RFI) and flavor precursor (nucleotides content), biochemical compound in thigh were investigated by High Performance Liquid Chromatography (HPLC) and Fourier Transform Infrared (FTIR) spectroscopy, respectively. The results showed FCR at 10 weeks of age was negatively correlated with Adenosine monophosphate (AMP) and inosine while RFI at 10 weeks of age was positively correlated with Inosine monophosphate (IMP). This indicated that improving FCR could impact on water

retention ability in the breast and thigh. Improving FCR and RFI could affect the flavor precursor in thigh.

The second study aim was to investigate the pathways and genes involved in feed efficiency and meat quality in KR chicken thigh by total RNA extraction from a total 21 of high FCR and low FCR. The microarray technique was used to detect gene expression data. Weighted gene coexpression network analysis (WGCNA) was used to investigate pathways and genes involving feed efficiency and meat quality. The results indicated that mitochondrial gene expression, mitochondria respiratory chain complex assembly, mitochondrial translation and positive regulation of mitochondrial translation pathways as well as the 4 genes: ACD, BIRC5, COA3, MYL9 might play a crucial role in feed efficiency and meat quality in slow-growing thigh muscle.



School of Animal Technology and Innovation Student's Signature

Academic Year 2018 Advisor's Signature

Co-advisor's Signature KANJANA THYMANU

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Chotima Poompramun

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# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| ADG | = | Average daily gain |
| AMP | = | Adenosine monophosphate |
| ATP | = | Adenosine triphosphate |
| BW | = | Body weight |
| BWG | = | Body weight gain |
| FCR | = | Feed conversion ratio |
| FI | = | Feed intake |
| FTIR | = | Fourier Transform Infrared |
| GMP | = | Guanosine monophosphate |
| g | = | Gram |
| HFCR | = | High feed conversion ratio |
| HRFI | = | High residual feed intake |
| IMP | = | Inosine, inosine-5’-monophosphate |
| kg | = | Kilogram |
| LFCR | = | Low feed conversion ratio |
| LRFI | = | Low residual feed intake |
| RFI | = | Residual Feed Intake |
| SE | = | Standard error |
| % | = | Percentage |

# CHAPTER I

# INTRODUCTION

## 1.1 Rationale of the study

Korat (KR) chicken is a new crossbred chicken from Thai indigenous Leung Hang khao chicken and SUT (Suranaree University of Technology) synthetic line. It was established with the purpose of developing Small and Micro Community Enterprise (SMCE) Production in order to promote farmer occupation, to ensure food security in communities, and to contribute to preservation of indigenous chicken breeds. The slow-growing KR chicken is usually slaughtered at 10 weeks of age with average body weight 1,200 g and consumed as grilled chicken. Its meat is recognized for its good texture, unique flavor, low fat and high collagen level (Katemala et al. 2018).

Consumer's demand has increasingly concerned dietary health (i.e., flavor, high protein digestibility, low fat). Currently, trend of slow-growing chicken has been growing because of its meat has good flavor and texture (Evans, 2015; Harris, 2011; Rikimaru and Takahashi, 2010). The feed efficiency in slow-growing chicken is represented by Feed conversion ratio (FCR) which its FCR range were reported from 2.94 to 4.66 (Hang et al., 2017; Liu et al., 2017; N'Dri et al., 2006; Jaturasitha et al., 2002). FCR of KR chicken at 84 days is 2.94 with 1,790 g (Hang et al., 2017), their FCR are higher than FCR of commercial broiler (FCR 2.14) (Jaturasitha et al., 2002). This is the significant weak point of slow-growing chicken leads to high cost of

production and low competitiveness especially in the current situation, where poultry feed industry is challenged by decreasing harvested area (decline 0.2% per year based on an average prediction over the period 2010-2060), increasing feedstuffs price, climate change (Harris, 2016; Arial et al., 2013; Pirvutoin and Popescu, 2013; Alexandratos and Bruinsma, 2012; Donohue and Cunningham, 2009; Ellis et al., 2002). Therefore, the genetic improvement for feed efficiency is important to reduce the cost production and the environment impact of poultry production.

Feed efficiency in chicken can assess by feed conversion ratio (FCR) and residual feed intake (RFI). FCR is considered as the ratio between amount of feed intake (FI) and body weight gain (BWG) that results in the synchronous selection of FI and BWG (Aggrey et al., 2010). RFI is defined as the difference between observed feed consumption and consumption predicted calculated by the growth rate and mean body weight (BW). By dividing the total energy of production and poultry into growth energy and maintenance energy which selection RFI is to produce a chicken with low feed intake and high production (Yi et al., 2018; Liu et al., 2017).

Concerning the meat quality, flavor can determine by nucleotides content that included Guanosine monophosphate (GMP), Inosine monophosphate (IMP), Adenosine triphosphate (ATP), Adenosine monophosphate (AMP), Inosine and Hypoxanthine that were detected by High Performance Liquid Chromatography (HPLC) technique). Texture and nutrients value can identify by technological quality (pH, water holding capacity (WHC) and drip loss) and biochemical compound (i.e., lipid, secondary protein structure consist α-helix structure and β-sheet, carbohydrate) respectively (Jayasena et al., 2013; Joo et al., 2013; Yu, 2005). Fourier transform infrared (FTIR) spectroscopy method was applied to detect the biochemical compound in the meat

based on vibration of a molecule excited by IR radiation at a specific wavelength range (Kirschner et al., 2004) which the total composition in the samples, including lipid, proteins (secondary structure protein) and carbohydrates/ glycogen can be revealed.

Regarding the correlation between feed efficiency (FCR, RFI) and technological quality, previous studies reported that FCR was negatively correlated to lightness, drip loss, leg yield in chicken whereas positively correlated to ultimate pH, abdominal fat, intramuscular fat in breast and thigh (Paiva et al., 2018; Wen et al., 2018; N'Dri et al., 2006) and RFI was also positively correlated to abdominal fat but the correlation between RFI and technological quality is still not investigated in chicken. The results of chapter 3 can fulfill this gap. Additionally, the correlation between feed efficiency (FCR, RFI) and flavor precursor (nucleotides content), biochemical compound (secondary structure protein) are not elucidated in chicken which this study has answered in chapter 4. The results of both chapters can reveal feed efficiency criteria that not impact on economic traits of meat quality.

Nowadays, the consumers especially from East Asia, Mexico, India, Russia, Morocco being to appreciate the tastiness of thighs and drumstick (Willems, 2018). This indicated the big chance to distribute the thighs of KR chicken to Asia market. Previous studies had focused the pathways and gene expression between high RFI and low RFI in duodenal tissue of layer chicken and *Longissimus thoracis et lumborum* (LTL) muscle in procine, respectively (Horodyska et al., 2018; Yi et al., 2015). Moreover, the pathways and genes associated intramuscular fat in breast slow-growing chicken (Chinese domestic Gushi chicken) was revealed by Li et al. (2019). The molecular information involved pathways and genes associated feed efficiency and

meat quality in thigh muscle slow-growing chicken are not explored. This study revealed the pathways and genes associated feed efficiency and technological quality, flavor indicators, biochemical compound in thigh muscle in chapter 5. As mentioned, the first objective of this study was to evaluate relationship between feed efficiency and meat quality that the results can be assessed the direction and limitation to improve the feed efficiency and texture, flavor, nutrients value in meat chicken. The second aim was to investigate pathways and genes involved in feed efficiency and meat quality traits in KR chicken useful to deeper understand molecular key that point the variation in feed efficiency and meat quality in chicken.

## 1.2 Research Objectives

1.2.1 To investigate the correlations between feed efficiency and meat quality in KR chicken.

1.2.2 To find out the pathways and the genes involved in feed efficiency and meat quality in thigh muscle of KR chicken.

## 1.3 Research hypothesis

Different feed efficiency (FCR, RFI) in chicken and sex are associated with variation of meat quality which are subjected to investigate the correlations between feed efficiency and meat quality in male KR chicken. This would lead to different meat quality upon gene expression profile, pathways involving feed efficiency and meat quality between high feed efficiency and low feed efficiency in chicken. This lead to identify pathways and genes involved in feed efficiency and meat quality in male KR chicken.

## 1.4 Scope and limitation of the thesis

The male of KR chicken is represented for target population. At 0-10 week of ages, the birds were raised on individual cage under the same environmental and nutritional conditions. They were fed *ad libitum* using commercial feed that starter diet (21% protein), grower diet (19% protein), and finisher diet (17% protein) at 0-3, 4-6, and 7-10 weeks of age, respectively. At 10 weeks of age, the breast muscle and thigh muscle were collected to analyze ultimate pH, Water Holding Capacity, drip loss. While the nucleotides content and biochemical compound were only measured in thigh muscle. 12 extreme low FCR and 10 extreme high in thigh muscle were isolated a total RNA for microarray analysis.

## 1.5 Expected benefits

1.5.1 The results of correlations between feed efficiency and meat quality is revealed which can explain the direction to improve both traits together.

1.5.2 Revealing the pathways and genes involving feed efficiency and meat quality that information can assess limitation for selection and gene biomarker development.

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# CHAPTER II

# LITERATURE OF REVIEW

## 2.1 Korat chicken (KR) qualification

Korat chicken (KR) is a new crossbred from Leung Hang khao (LK) sire and SUT dam. KR chicken was established with the objectives of developing Small and Micro Community Enterprise (SMCE) production in order to promote farmer occupation, to ensure food security in communities, and to contribute to preservation of indigenous chicken breeds. The project is done under the cooperation between Thailand Research Fund (TRF), Suranaree University of Technology (SUT) and Department of Livestock Development (DLD). The meat of Korat chicken (KR) is recognized for unique tasty, good texture, low fat content and low purine contents reported by Katemala et al. (2019). KR chicken is a slow-growing chicken which is usually slaughtered at 10 weeks and consumed as grilled chicken. The average of feed conversion ratio (FCR) at 84 day is 2.85 with 1,582.51 g (Hang et al., 2017), that is higher than commercial broiler (FCR range from 1.44 to 1.46) reported by Dozier III and Gehring (2014), leads to high cost of production, and it is the significant weak point of the chicken. Genetic improvement for the trait, therefore is necessary. The improvement, however, any important must not negatively impact to the quality of meat that is the outstanding qualification of the chicken.

## 2.2 Trend of poultry consumption and animal feedstuffs price

The global poultry consumption and average of Asian eating have been increasing from 2000-2011 (Figure 2.1). The globally in 2011, poultry could uptake from 12.3 kg to 12.5 kg per person, while in Asia could be reached 7.2 kg from 7.0 kg (Evans, 2015; Harris, 2011).

Feedstuffs (e.g., corn, soybean) are main ingredients to produce animal feed. As shown in Figure 2.2 trend of the feed cost continues to increasing that due to world cereal price and Thailand feed ingredients have highly cost (Table 2.1, Figure 2.3). Also, decreasing harvested area, heat and lack of rainfall were important factors that effect on the abrupt price increase of feedstuffs (Alexandratos and Bruinsma, 2012). This indicated that the trend of climate change, limited of land to produce the feedstuffs lead to continue increasingly the feed cost in poultry industry. Some of studies indicated that improving feed efficiency could reduce cost production in poultry production (Table 2.2). Therefore, the selection feed efficiency trait in chicken is important trait to reduced feeding cost and environment impact in the poultry farming system.

**Table 2.1** Wholesale Prices for Feed Ingredients and Retail Chicken Meat in Thailand.

| **Details** | **2017 years** | **2018 years** |
|---|---|---|
| | **Average price** (Baht/kg) | **Average price** (Baht/kg) |
| Corn | 8.23 | 10.12 |
| Soybean | 14.81 | 15.57 |
| Boneless Breast | 74.55 | 68.16 |
| Leg Quarters | 76.25 | 68.52 |

Source Ward et al. (2016)

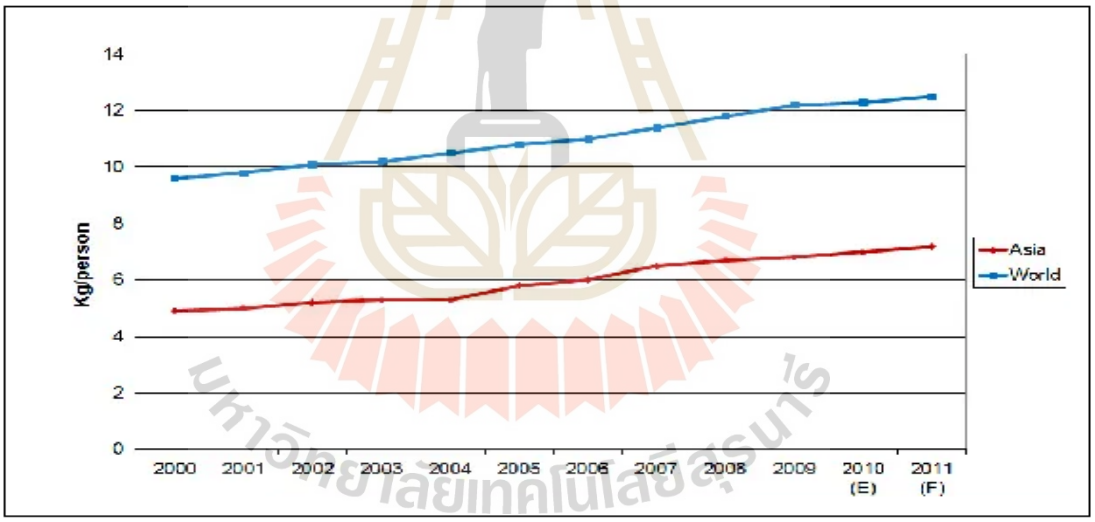


**Figure 2.1** Comparison poultry meat consumption in Asia and world average Source Harris (2011).

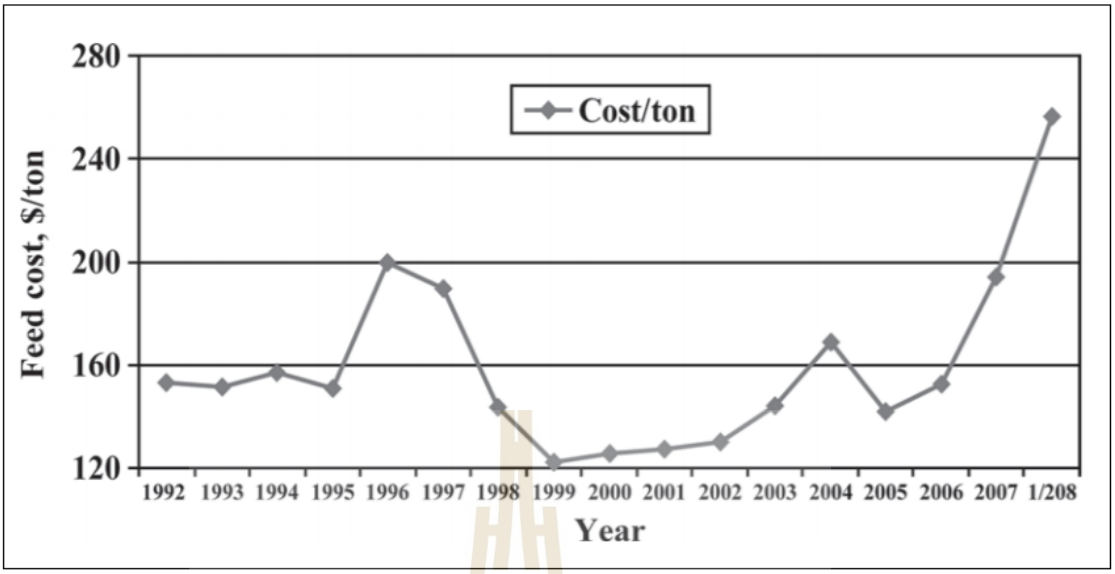


**Figure 2.2** Feed cost per ton for the US broiler industry Source Donohue and Cunningham (2009).

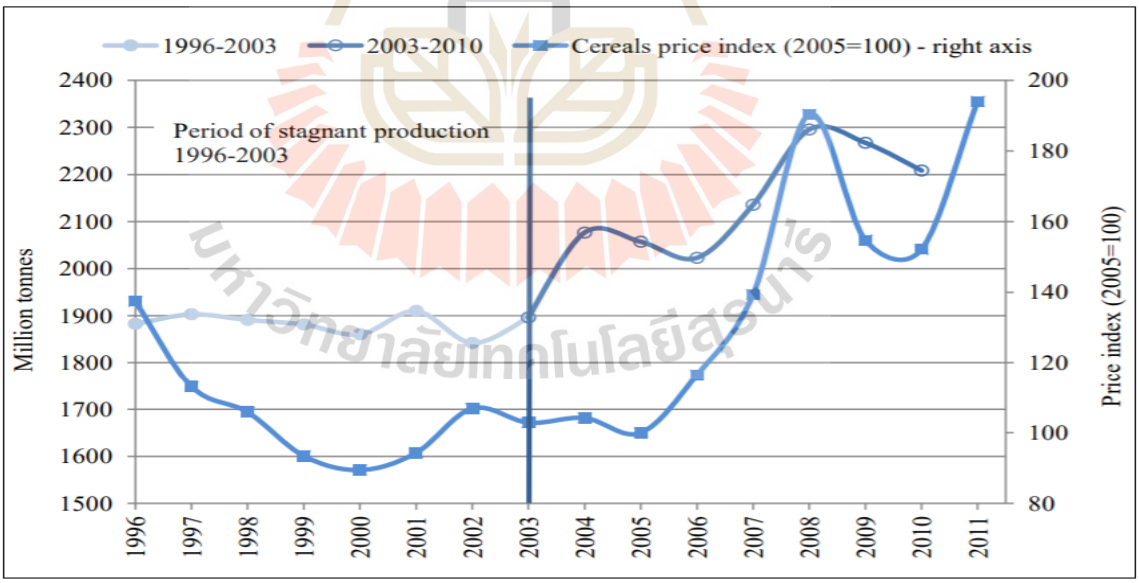


**Figure 2.3** World cereal production 1996-2010 (million tonnes) and prices Source Alexandratos and Bruinsma (2012).

**Table 2.2** Comparison cost production on different FCR in chicken.

| Breed | Slaughter weight | FCR | Cost production | References |
|---|---|---|---|---|
| Hubbard Red JA (raised 81 days) | 2,778 (g) | 2.93 | 3.03(€/kg) | Cobanoglu et al. (2014) |
| Ross-308 (raised 42 days) | 2,250 (g) | 1.93 | 1.62 (€/kg) | |
| (Broiler X layer) X Chee | - | 2.06[a] | 14.87[a](Baht/kg) | Promket et al. (2013) |
| (Shabghai Road Bar X Layer) X Chee | - | 2.44[b] | 21.24[b](Baht/kg) | |
| Thai native (raised 12 wks) | 1,556.05 (g) | 4.66[a] | 33.24[a](Baht/kg) | Jaturasitha et al. (2002) |
| Broiler (raised 6 wks) | 1,997.00 (g) | 2.14[b] | 30.99[b](Baht/kg) | |

## 2.3 Feed efficiency and growth performance in KR chicken

Feed efficiency is usually measured by the feed conversion ratio (FCR) and residual feed intake (RFI). FCR is defined as the ratio between feed intake and body weight gain. RFI is defined as difference between observed feed consumption and consumption expected necessary for maintenance and production (Izadnia et al., 2018). RFI represented the amount of FI not account for maintenance body weight and growth weight (Xu et al., 2014) that means the low RFI chicken could produce high production with low FI. The heritability of FCR and RFI in chicken were 0.41 and 0.49 respectively (Aggrey et al., 2010).

Table 2.3 showed among those chickens, the feed efficiency, body weight and growth curve of KR chicken used to be closed to the slow-growing chicken from France (Label Rouge chicken). While the commercial slow-growing chicken (Hubbard)

showed lower FCR and higher body weight than KR chicken. This indicated the low competitiveness in feed efficiency and growth performance of KR chicken especially in the current situation where poultry feed industry is confronted with high cost feed.

**Table 2.3** Feed efficiency and growth performance in chicken.

| **Breed** | **Growth performance** | | | | **Growth curve** | | | **References** |
|---|---|---|---|---|---|---|---|---|
| | FCR | RFI (g) | BW (g) | ADG (g/day) | L ($d^{-1}$) | K ($d^{-1}$) | TI ($d^{-1}$) | |
| Korat (70 days) | 2.62 | -1.62 | 1460.00 | 20.22 | 0.12 | 0.03 | 47.04 | This study |
| At 42 days | | | | | | | | |
| Cobb | - | - | 4252.00 | - | - | 0.05 | 33.88 | Demuner |
| Ross | - | - | 4400.00 | - | - | 0.05 | 34.44 | et al. |
| Hubbard | - | - | 4220.00 | - | - | 0.04 | 36.01 | (2017) |
| Korat (84 days) | 2.94 | - | 1790.00 | 24.13 | | | | Hang et al. (2017) |
| China crossbred (42 days) | 3.54 | 0.00 | - | 29.34 | - | - | - | Liu et al. (2017) |
| At 70 days | | | | | | | | |
| Shaobo | - | - | 1319.93 | 21.84 | 0.12 | - | 60.48 | Zhao et al. |
| Huaixiang | - | - | 800.53 | 12.90 | 0.16 | - | 51.66 | (2011) |
| Youxi | - | - | 1202.46 | 17.00 | 0.13 | - | 59.15 | |

**Table 2.3** Continue.

| Breed | Growth performance | | | | Growth curve | | | References |
|---|---|---|---|---|---|---|---|---|
| At 84 days | | | | | | | | |
| Ross | 2.08 | - | 3224.00 | - | - | - | - | Yamak et |
| Crossbred | 2.37 | - | 1946.00 | - | - | - | - | al. (2014) |
| Hubbard | 2.42 | - | 2101.00 | - | - | - | - | |
| Label Rouge | - | - | 1973.00 | - | 0.14 | 0.03 | 49.70 | N'Dri et al. (2007) |
| At 21 weeks | | | | | | | | |
| Naked neck | - | - | 2231.61 | - | 0.17 | - | 57.61 | Norris et |
| Venda | - | - | 2240.13 | - | 0.14 | - | 67.2 | al. (2007) |
| Label Rouge | 3.15 | 0.62 | 1971.00 | - | 0.14 | 0.03 | 48.90 | N'Dri et al. (2006) |
| Thai native | 4.66 | - | 1156.05 | 16.56 | - | - | - | Jaturasitha |
| Broiler | 2.14 | - | 1997.00 | 57.90 | - | - | - | et al. (2002) |

L = initial specific growth rate; K = maturation rate; TI = age at inflexion, FCR = feed conversion ratio, RFI = residual feed intake, BW = body weight, Thai native (slaughterd at 12 weeks of age), Broiler (slaughterd at 42 days of age)

## 2.4 Meat quality in chicken

### 2.4.1 Meat quality parameters in chicken

Meat quality is affected by extrinsic factors (i.e., feeding, age, sex, slaughter process, postmortem process), intrinsic factors (i.e., genetics, hormone regulation, muscle fiber type structure) and interaction between genetic and environment (Diarra and Tabuaciri, 2014; Wood and Willems, 2014; Joo et al., 2013;

Tougan et al., 2013; De Liu et al., 2012; N'Dri et al., 2007). Chicken meat quality can be defined from appearance quality traits and eating quality traits (Figure 2.4).

### 2.4.2 Decline pH in muscle link to water retention ability

Skeletal muscle consists of approximately 75% water, 20% protein, 1-10% fat, and 1% glycogen (Listrat et al., 2016). In living muscle tissue, pH is about 7.4 which it used energy by aerobic and anaerobic pathways. When the chicken is dead the muscle tissues shift their ATP production (energy) from aerobic to anaerobic resulted that cannot sustain normal muscle energy need. The absence of ATP led to release calcium (a rise in ionic strength) from the sub-cellular sarcoplasmic reticulum (SR) and the muscle proteins start to form cross-bridges called rigor mortis. Additionally, the lactic acid is increased by the anaerobic metabolism of glycogen and the pH start to decline. The normal pH value after rigor mortis is 5.5 to 5.7 (Matarneh, 2017).

Ultimate pH decline has an influence on the proteolysis (i.e., l-calpain form, m-calpain form degradation) may cause protein conformational changes that allow to release the water from the intramyofibrillar spaces related to drip loss in meat (Huff-Lonergan and Lonergan, 2005). Ultimate pH was positively related to water holding capacity, drip loss, intra muscular fat while negatively related to water, colour, glycolytic potential in meat (Watanabe et al., 2018; Le Bihan-Duval et al., 2008; El Rammouz et al., 2004; Qiao et al., 2001). Moreover, higher and lower pH related to Pale Soft Exudative (PSE) and Dark Firm Dry (DFD) in meat (Lesiów and Kijowski, 2003). This indicated alteration ultimate pH is important to water retention ability (tenderness), biochemical compound (protein, intramuscular fat) in meat.

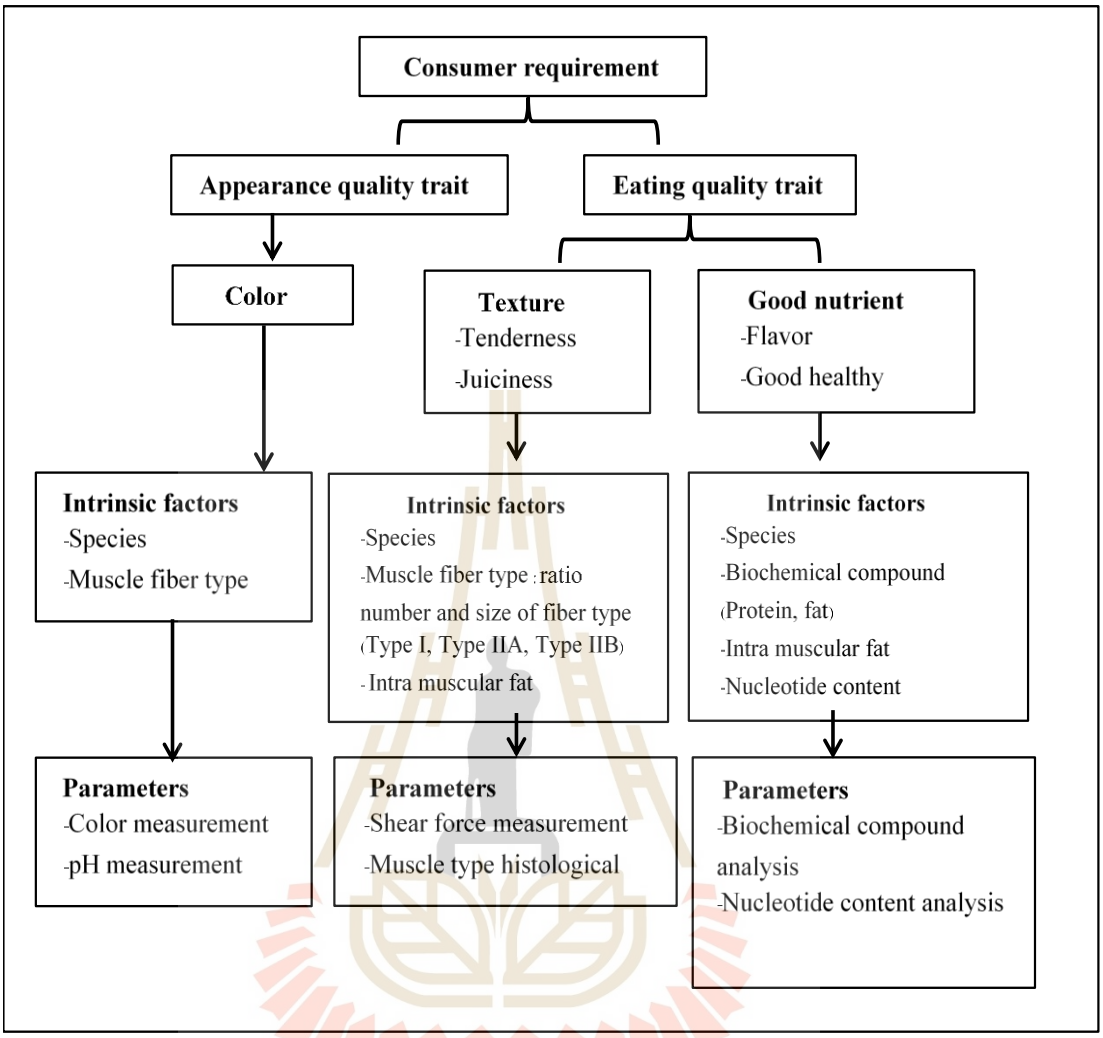


**Figure 2.4** Meat quality traits link with meat parameters in fresh meat (applied from Joo et al., 2013)

### 2.4.3 Nucleotides content

Flavor composed mainly of taste and aroma and is involving in consumer's meat buying and preferences. The characteristic of fresh meat is bloody, metallic, salty taste and its aroma appear like blood serum (Jayasena et al., 2013a). Meat flavor formation and development can assess by Inosine monophosphate (IMP) and its degradation products, Guanosine 5'-monophosphate (GMP) and hypoxanthine.

IMP is produced by three pathways, de novo synthesis, salvage pathway and IMP transformation (Tikk et al., 2006; Heath, 1970). IMP is a product of adenosine triphosphate (ATP) via enzymatic hydrolysis after slaughter. ATP degradation produces a series of compounds: ATP to adenosine diphosphate (ADP) to adenosine monophosphate (AMP) to IMP (Kavitha and Modi, 2007). This compound is serially transformed into inosine, ribose and hypoxanthine in the meat.

Based on literatures, the concentration of IMP in slow-growing chickens were higher compared to broiler chicken (Youngsawadigul et al., 2016; Guan et al., 2013; Rikimaru and Takahashi, 2010) while the concentration of GMP in broiler was higher than slowin-growing chicken. In addition, the concentration of IMP in breast had higher than thigh. This might explain by the different composition of muscle fibers type, muscle fibers number and muscle fibers diameter in breast and thigh which the breast muscle composed mostly muscle fiber type IIB (glycolytic muscle fiber) (Tůmová and Teimouri, 2009). While the thigh is mainly composed of the muscle fiber type I (oxidative muscle fiber) and the different ratio of muscle fiber type I, type IIA, type IIB depended on breed (Jaturasitha et al., 2008). Moreover, the muscle fibers diameter was negatively correlated to IMP while positively to muscle fibers number (Yang et al., 2011). This could hypothesize that the chicken with high feed efficiency and low feed efficiency might contain the different nucleotides content in meat.

### 2.4.4 Meat quality in KR chicken

Consumers have become increasingly concerned about dietary health, flavor and texture (Arial et al., 2013; Pirvutoin and Popescu, 2013; Ellis et al., 2002). Indigenous chicken meat has become to be attracted trend in consumer because its meat is good flavor and texture (Lee et al., 2017; Jayasena et al., 2013b; Zhao et al.,

2011). Nowadays, the meat quality of KR chicken is accepted by consumers. Parameters meat quality in KR chicken were closely indigenous chicken as showed in Table 2.4. Therefore, tradeoff between feed efficiency, growth and meat quality traits is an important issue for current and future breeding plans in KR chicken.

**Table 2.4** The situation of meat quality in chicken.

| Meat quality parameters | Breed | Body weight (g) | References |
|---|---|---|---|
| **Breast** | **Korat (10 weeks)** | - | |
| Moisture (%) | 75.80* | | |
| Crude Protein (% dry) | 90.84* | | |
| Crude fat (% dry) | 3.59* | | |
| **Thigh** | | | |
| Moisture (%) | 76.71* | | |
| Crude Protein (% dry) | 81.74 | | Katemala et al. |
| Crude fat (% dry) | 10.57* | | (2019) |
| **Breast** | **Broiler (6 weeks)** | - | |
| Moisture (%) | 76.91* | | |
| Crude Protein (% dry) | 84.76* | | |
| Crude fat (% dry) | 5.95* | | |
| **Thigh** | | | |
| Moisture (%) | 75.41* | | |
| Crude Protein (% dry) | 75.36 | | |
| Crude fat (% dry) | 16.77* | | |
| | **Leung Hang Khao** | 1457.60 | |
| **Breast** | **(6 weeks)** | | |
| Drip loss (%) | 2.64 | | Molee et al. |
| Shear force (g/mm) | 2.18 | | (2018) |
| **Thigh** | | | |
| Drip loss (%) | 148.96 | | |
| Shear force (g/mm) | 108.59 | | |

**Table 2.4** Continue.

| Meat quality parameters | Breed | Body weight (g) | References |
|---|---|---|---|
| **Breast** | **Korat (84 days)** | 1582.51 | |
| Ultimate pH | 6.71 | | |
| Drip loss (%) | 10.51 | | |
| SFA (g/100 total fatty acid) | 31.96 | | |
| n-6 (g/100 total fatty acid) | 35.41 | | |
| n-3 (g/100 total fatty acid) | 2.08 | | Hang et al. (2018) |
| n-6/n-3 (g/100 total fatty acid) | 17.24 | | |
| **Thigh** | | | |
| Drip loss (%) | 7.76 | | |
| SFA (g/100 total fatty acid) | 26.93 | | |
| n-6 (g/100 total fatty acid) | 35.88 | | |
| n-3 (g/100 total fatty acid) | 1.62 | | |
| n-6/n-3 (g/100 total fatty acid) | 22.23 | | |
| **Breast** | **Korat (12 weeks)** | 1530.00 | |
| GMP (µg/g) | 22.46 | | |
| IMP (µg/g) | 800.90 | | Youngsawadigul et al. (2016) |
| **Thigh** | | | |
| GMP (µg/g) | 11.40 | | |
| IMP (µg/g) | 239.64 | | |
| **Breast** | **Broiler (5 weeks)** | 1550.00 | |
| GMP (µg/g) | 27.51 | | |
| IMP (µg/g) | 619.35 | | |
| **Thigh** | | | |
| GMP (µg/g) | 23.42 | | |
| IMP (µg/g) | 581.53 | | |

**Table 2.4** Continue.

| Meat quality parameters | Breed | Body weight (g) | References |
|---|---|---|---|
| | **Thai indigenous (16 weeks)** | 1280.00 | |
| **Acceptability** | 6.67 | | |
| **Breast** | | | |
| Ultimate pH | 5.77 | | |
| Drip loss (%) | 6.39 | | |
| Moisture (%) | 72.9 | | |
| Crude Protein (% dry) | 91.14 | | |
| Crude fat (% dry) | 1.88 | | |
| **Thigh** | | | |
| Drip loss (%) | 3.42 | | |
| Moisture (%) | 75.7 | | |
| Crude Protein (% dry) | 83.95 | | Jaturasitha et al. |
| Crude fat (% dry) | 12.10 | | (2008) |
| | **Shanghai** (16 weeks) | 1700.00 | |
| **Acceptability** | 7.17 | | |
| **Breast** | | | |
| Ultimate pH | 5.71 | | |
| Drip loss (%) | 6.45 | | |
| Moisture (%) | 73.3 | | |
| Crude Protein (% dry) | 89.51 | | |
| Crude fat (% dry) | 2.21 | | |
| **Thigh** | | | |
| Drip loss (%) | 3.97 | | |
| Moisture (%) | 74.5 | | |
| Crude Protein (% dry) | 77.65 | | |
| Crude fat (% dry) | 21.76 | | |

## 2.5 Correlation between feed efficiency and meat quality in chicken

### 2.5.1 Correlation of feed efficiency and phenotypic meat quality

As shown in Table 2.5 indicated that improving feed efficiency (FCR) could be decreasing the lightness, water retention ability, fat in breast muscle while RFI was reduced breast and thigh yield. Low FCR chicken correlated with high body weight (Wen et al., 2018). The chicken with high body weight had greater diameter of muscle type IIA and type IIB but smaller type I in thigh muscle (Jaturasitha et al., 2008). The study of Ryu and Kim, (2005) indicated that high percentage of muscle fibers type IIB in meat could be an effect on low pH and much drip loss. While percentage muscle fibers type IIA was positively and negatively with ultimate pH and drip loss respectively. This could be hypothesized that the high FCR and low FCR might has differentially of muscle fiber type structure in meat. Moreover, Yu (2005) reported that high protein values involving the ratio of secondary structure protein (α-helix structure and β-sheet structure) that high percentage of β-sheet structure in meat might be indicated low protein digestibility because low access to gastrointestinal digestive enzymes. Base on evidences indicate that the phenotypic correlation between feed efficiency and flavor, biochemical compound are still not explored in chicken. Therefore, the first objective in this study was to fulfill this information.

### 2.5.2 Mechanism involved in feed efficiency and meat quality

The mechanism involved in feed efficiency and meat quality in chicken is not clearly. High feed efficiency (low FCR) chicken related to more total feed intake and high body weight (Wen et al., 2018). Previous study reported the different expression mRNA of IGF-1 and IGF-2 in high growth performance and low growth performance chicken (Giachetto et al., 2004; Beccavin et al., 2001). In addition,

Growth Hormone (GH), Insulin like-growth factor 1 (IGF-1), Insulin like-growth factor 2 (IGF-2), Thyroid Hormone (TRH), Triiodothyronine (T3), Tyroxine (T4) were associated with growth performance and muscle development in chicken (Kim, 2010) as showed in Figure 2.5.

**Table 2.5** Correlation between feed efficiency and meat quality in chicken.

| **Meat parameters** | **Coefficient of feed efficiency** | | **References** |
|---|---|---|---|
| | **FCR** | **RFI** | |
| **Breast broiler** (genetic correlation) | | | |
| Initial pH | -0.30 | - | |
| Ultimate pH | 0.33 | - | |
| Lightness | -0.30 | - | Paiva et al. (2018) |
| Drip loss | -0.49 | - | |
| Thawing loss | -0.23 | - | |
| Shear force | -0.50 | - | |
| **Slow-growing broiler** (Phenotypic correlation) | | | |
| Abdominal fat weight | -0.11 | 0.41** | Wen et al. (2018) |
| Intramuscular fat content of breast | 0.16* | 0.13 | |
| Intramuscular fat content of thigh | 0.15* | 0.07 | |
| **Label Rouge** (genetic correlation) | | | |
| Breast yield | -0.00 | -0.35 | N'Dri et al. (2006) |
| Leg yield | -0.70 | -0.32 | |
| Abdominal fat yield | 0.44 | 0.44 | |

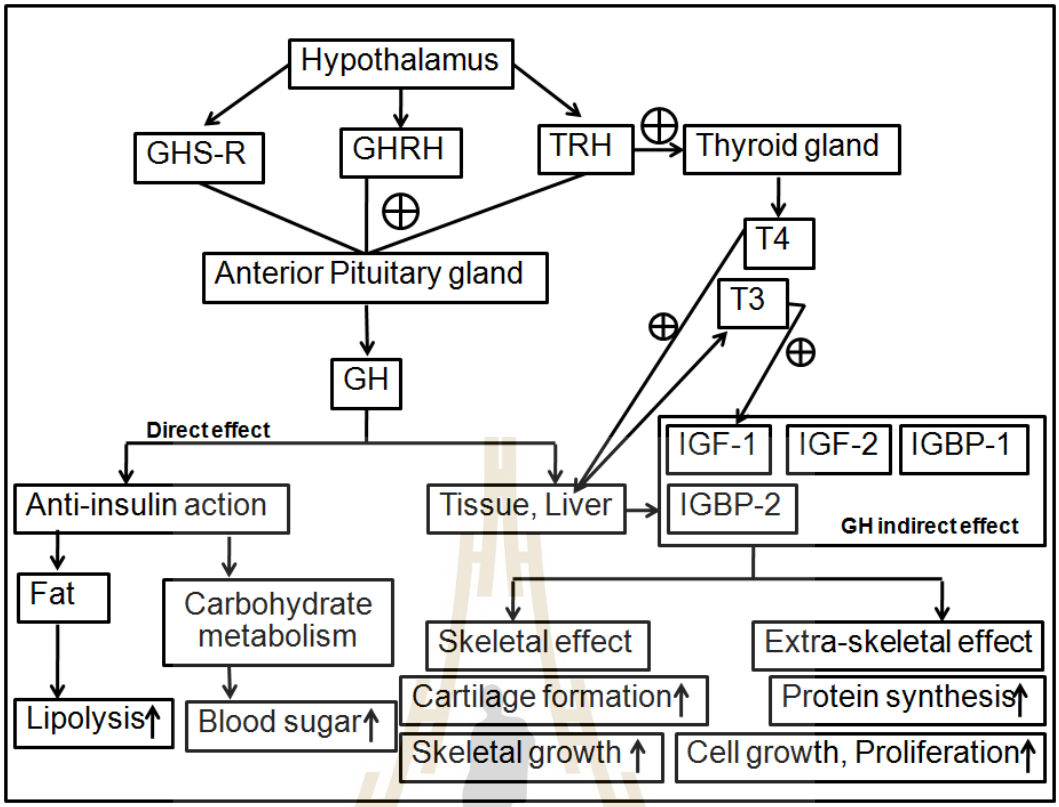


**Figure 2.5** Regulation of hormone on growth performance and muscle development.

### 2.5.3 Pathways and genes involved in feed efficiency and meat quality in chicken

Nowadays, the whole legs (thighs, drumsticks) are requirement by consumers such as East Asia, Mexico, India, Russia, Morocco are preferring this product (Willems, 2018). Based on literatures, the broiler with high feed efficiency had mostly up-regulated genes in breast muscle associated with inflammatory response and free radical scavenging (Zhou et al., 2015) and was also reported the 41 genes were significantly differently expressed between high RFI and low RFI in layer chicken which genes involved in digestibility, metabolism and energy homeostasis in duodenal tissue (Yi et al., 2015). Moreover, the expression of microRNA (miRNA) in breast of high feed efficiency and low efficiency was differentially expressed their

associated with calcium signaling, axonal guidance signaling, and NRF2-mediated oxidative stress response pathways (Khatri et al., 2018). The pathways and genes involved in feed efficiency and meat quality in thigh chicken is still not elucidated in chicken. Therefore, the second in this study was to investigate genes and molecular pathways those may involve in feed efficiency and meat quality in slow-growing KR chicken.

## 2.4 Fourier transform infrared (FTIR)

The technique is the method of infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it passed through. The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. The process of absorption and transmission, with the specific wavelengths are absorbed causing the chemical bonds between atom in the sample to undergo vibrations such as stretching, contracting, and bending (Davis and Mauer, 2010). Therefore, each different sample is a unique combination of atoms, no two compounds produce the exact same infrared spectrum. Infrared spectroscopy can result in a positive identification of every different kind of sample. In addition, the size of peaks in the spectrum is a direction of the amount of sample present on Table 2.6. From the review of Davis and Mauer (2010), they reported the advantages and disadvantages of FTIR technique for analyzing microorganisms. The advantages of the technique are, 1) relatively fast and simple to use, little or no sample preparation required for spectral acquisition. 2) sensitivity method that requires very little sample. 3) nondestructive 4) qualitative as well as quantitative analysis 5) multiple sample environment. The

disadvantages are the environmental conditions around the FTIR instrument can cause variation in the spectra, hence background scans and multiple scans of the same sample are required. The several studies on table 10 can ensure that FTIR technique can apply for this study.

**Table 2.6** Relationship between functional group and wave number.

| Wave number (cm-1) | Molecular vibrations of functional groups | Functional groups marke | biomolecule contributor | References |
|---|---|---|---|---|
| 3200 | N-H stretching of amide A | N-H | proteins | |
| 2955 | C-H asymmetric stretching of -CH3 | C-H | fatty acids | |
| 2930 | C-H asymmetric stretching of >CH2 | C-H | fatty acids | |
| 2898 | C-H stretching of ≥C-H | C-H | Amino acids | |
| 2870 | C-H symmetric stretching of -CH3 | C-H | fatty acids | Santos et al. (2015) |
| 2850 | C-H symmetric stretching of >CH2 | C-H | fatty acids | |
| 1740 | >C=O stretching | C=O | lipid esters | Davis and Mauer (2010) |
| 1715 | >C=O stretching of ester | C=O | nucleic acids and carbonic acids | |
| 1695-1675 1600-1690 | Amide I band | C=O | components of proteins | |
| 1655 | Amide I | C=O | α-helical structures of proteins | |
| 1637 | Amide I | C=O | β-pleated sheet structures of proteins | |
| 1550-1520 1480-1575 | Amide II band | C-N, N-H | proteins | |
| 1515 | Tyrosine band | | | |
| 1468 | C-H deformation of >CH2 | C-H | lipids proteins | |

**Table 2.6** Continue.

| Wave number (cm-1) | Molecular vibrations of functional groups | Functional groups marke | biomolecule contributor | References |
|---|---|---|---|---|
| 1415 | C-O-H in-plane bending | C-O-H | Carbohydrates, DNA/RNA backbone, proteins | |
| 1400 | C=O symmetric stretching of COO- group | C=O | Amino acids, fatty acids | |
| 1310-1240<br>1229-1301 | Amide III band | C-N, N-H | proteins | |
| 1240 | P=O asymmetric stretching | P=O | phosphodiesters in phospholipids | |
| 1200-900 | C-O-C, C-O dominated by ring vibrations | C-O-C, C-O | polysaccharides | |
| 1085 | P=O symmetric stretching | P=O | DNA, RNA and phospholipids | |
| 720<br>900-600 | C-H rocking of >CH2 | C-H | fatty acids, proteins<br>"Fingerprint region" | |

## 2.5 References

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# CHAPTER III

# INTERACTION BETWEEN FEED EFFICIENCY, GROWTH AND MEAT QUALITY OF SLOW-GROWING CHICKEN: PHENOTYPIC CHARACTERIZATION OF NATIVE KORAT CHICKEN

## 3.1 Abstract

Korat (KR) chicken is a new alternative meat-type chicken breed established with the purpose of developing Small and Micro Community Enterprise Production. This slow-growing chicken is usually slaughtered at 10 weeks of age and is recognized for its good texture and flavor. However, its low feed efficiency leads to high cost of production and low competitiveness especially in the current situation where poultry feed industry is confronted with increasing cost of feedstuffs. This highlights the importance of studying the tradeoff between feed efficiency and meat quality in KR chicken.

The objective of this study was to investigate phenotypic correlations between feed efficiency, growth performance, and meat quality by using 75 male KR chickens reared up to 10 weeks. Individual body weight and feed intake were recorded weekly for the calculation of the Feed Conversion Ratio (FCR) and the Residual Feed Intake (RFI). Growth curve was modelled by Gompertz function and meat quality evaluated

at 10 weeks through the measurement of ultimate pH (pHu), water-holding capacity (WHC) and drip loss (DL) in breast and thigh muscle.

The initial specific growth rate (L), maturation rate (K) and age at inflexion (TI) of KR chicken used to be close to the mean values reported on Label Rouge (LR) chickens, indicating that the two lines had similar shape of growth curve. However, BW at slaughter was lower for KR than for LR chickens. Faster growth rate at young age appeared favorable regarding FCR since moderate negative correlation was found between the latter trait and L parameter (from -0.07 to -0.41 according to the age). Our results also suggested that chickens with higher initial growth rate and maturation rate were characterized by a lower water-holding capacity of the meat. This did not seem to be associated with a propensity to develop more acid meat, since a higher speed of growth (especially in the first weeks) was positively associated with ultimate pH, at least in breast meat. The results of the current study suggested that improvement of FCR could have a detrimental effect on water-retention of breast and thigh meat, while the relationship between RFI and meat quality traits was weak, suggesting that the latter trait could be an alternatively criteria of interest.



## 3.2 Introduction

Korat (KR) chicken is a new alternative meat-type chicken breed which is a crossbred between Thai indigenous Leung Hang khao chicken and a SUT (Suranaree University of Technology) synthetic line. It was established with the purpose of developing Small and Micro Community Enterprise (SMCE) production in order to promote farmer occupation, to ensure food security in communities, and to contribute to preservation of indigenous chicken breeds. The slow-growing KR chicken is

usually slaughtered at 10 weeks of age and consumed as grilled chicken. It is recognized for its high protein, low fat and low purine content (Katemala et al., 2018). However, the low feed efficiency of KR chicken leads to high costs of production and low competitiveness especially in the current situation where poultry feed industry is confronted with variation in cost of feedstuffs (Donohue and Cunningham, 2009; Mittal, 2009).

The efficiency of the birds to utilize feed is usually estimated by the feed conversion ratio (FCR) or the residual feed intake (RFI) (Case et al., 2012; Zuidhof et al., 2014). FCR is defined as the feed intake per unit of body weight gain, and RFI by the difference between observed feed consumption and consumption predicted from multiple regression on metabolic body weight and body weight gain. Feed efficiency can be efficiently selected as highlighted by significant heritability of FCR and RFI in meat-type chicken (Aggrey et al., 2010; Liu et al., 2017). The genetic relationships between feed efficiency and growth or carcass traits in the context of slow-growing chickens was studied by N'Dri et al. (2006). Moderate correlations were evidenced between FCR or RFI and some of the growth curve parameters, revealing that in this population birds with a higher initial growth rate were less efficient. Results confirmed a negative genetic relationship between fatness and feed efficiency, as well as improved feed efficiency in birds with higher breast or leg proportion, although correlations were more or less pronounced according to the criteria (i.e., FCR or RFI). Regarding meat quality, weak but unfavorable phenotypic relationships were evidenced between intramuscular fat content (in thigh muscle) and FCR in slow-growing chickens (Wen et al., 2018). Unfavorable genetic relationships were also suggested between FCR and indicators of the sensorial and technological quality of

the meat, such as lightness and water retention ability, measured in a fast-growing strain (Paiva et al., 2018).

Tradeoff between feed efficiency, growth and meat quality traits is an important issue for current and future breeding plans in meat-type chickens. Based on the literature, it seems that this balance may vary depending on several factors such as the genetic background or the stage of development. Therefore, the aim of this study was to investigate the correlations between FCR, RFI, growth curve parameters, BW measured weekly and meat quality parameters in slow-growing KR chicken.

## 3.3 Materials and methods

### 3.3.1 Ethics Statement

The experiment was conducted at the experimental farm of the Suranaree University of Technology (SUT). All procedures used in this study were approved by the Ethics Committee on Animal Use of the Suranaree University of Technology, Nakhon Ratchasima, Thailand, whose document ID is U1-02631-2559.

### 3.3.2 Experimental animals and data collection

A total of 75 male slow-growing KR chickens produced in three batches were used. At hatch, birds were sexed, wing-banded, vaccinated against Marek's disease and coccidiosis disease and individually weighed. All KR chickens were raised in individual cage in the same environmental and nutritional conditions. They were fed ad libitum using starter diet (21% protein), grower diet (19% protein), and finisher diet (17% protein) at 0-3, 4-6, and 7-10 weeks of age, respectively. Individual body weight and feed consumption were recorded weekly from 1 week to 10 weeks of age, for the calculation of FCR and RFI.

### 3.3.3 Meat quality traits measurements

At 10 weeks and after 8 hours of fasting, chickens were transported to the slaughterhouse, then rested for 30 min and killed by neck cutting for bleeding. After slaughter, carcasses were washed with clean water and held at 4°C for 24 h before cutting into portions.

The ultimate pH (pHu) of breast and thigh was measured 24 h postmortem with a portable pH-meter (pHCore-kit, Satorius Lab Instruments GmbH, Goettingen, Germany) by direct insertion of its glass electrode into the muscle after calibration using buffers (pH 4.01 and 7.00) at room temperature.

The percentage of drip loss (DL) was measured from raw meat samples weighing approximately 4-5 g, cut into pieces with dimensions of approximately 1.0 × 2.0 × 0.5 cm (width, length, and height, respectively). The samples were wrapped in absorption pads, placed in polyethylene bags, stored for 24 h at 4°C and weighed again to calculate drip loss (DL) as follows:

$$DL\ (\%) = 100 \times \frac{Weight_{\text{before storage}} - Weight_{\text{after storage}}}{Weight_{\text{before storage}}}$$

The water-holding capacity (WHC) index of meat samples was determined according to a method based on Sakata et al. (1993). Individual breast or thigh meat was chopped and weighed (5 g). After that, the sample was placed on nylon net and wrapped with 3 pieces of filter paper (Whatman No. 4). The wrapped sample was centrifuged at 3000 x g for 20 min (Thermo Fisher Scientific, Langenselbold, Germany) to calculate water-holding capacity (WHC) as follows:

$$WHC\ (\%) = 100 \times \frac{Meat\ weight_{\text{after storage}}}{Meat\ weight_{\text{before storage}}}$$

### 3.3.4 FCR and RFI calculation

FCR in week J (FCRj) was calculated as the ratio between feed intake from hatch to week j and body weight gain over the same period. The Residual Feed Intake in week j ($RFI_j$) was calculated weekly as follows:

$$RFI_j = \text{Total feed intake for week j (g)} - (b_0 + b_1\, MMW_j + b_2\, BWG_j)$$

where $BWG_j$ is the body weight gain between week j and j-1 (g), $MMW_j$ the metabolic weight estimated from mean body weight at week j and j-1 (i.e.,$\left(\frac{Bw_j - Bw_{j-1}}{2}\right)^{0.75}$), $b_0$ is the intercept, and $b_1$ and $b_2$ are partial regression coefficients. Then we considered cumulative RFI from hatch to week J (CUMRFIj) as

$$CUMRFI_i = \sum_{i}^{j} RFI_i$$

### 3.3.5 Estimation of growth curve parameters

Gompertz model was used to estimate growth curve parameters according to Laird et al. (1965):

$$BW_t = BW_0\, x\, e^{\left(\frac{L}{K}\left(1-e^{-Kt}\right)\right)}$$

where BWt is the body weight at age t, BW0 the estimated weight at hatching, L the initial specific growth rate $\left(\frac{1}{BW_t}\, x\, \frac{dW_t}{d_t}\right)$, and K the maturation rate or the exponential factor of decay of the specific growth rate. The calculation of the age at inflexion (TI), for which the growth rate is maximum, was as follows:

$$TI = \left(\frac{1}{k}\right) In \left|\frac{L}{K}\right|$$

The growth curve parameters were estimated by non-linear regression with the NLIN procedure of SAS (SAS Institute, 1999) taking into account all available weights from birth to slaughter. Body weights at each age were weighted by the ratio of phenotypic variance of slaughter weight to phenotypic variance of $BW_t$, in order to take into account the increase of variance of body weight with age.

### 3.3.6 Correlation analysis

Pearson correlations between FCR, RFI, BW at the different ages, and parameters of growth curve and meat quality traits were calculated using the proc corr procedure from SAS (SAS Institute, 1999). Estimates with P-value<0.05 were considered significant.

## 3.4 Results and discussion

### 3.4.1 Phenotypic correlations between growth and feed efficiency parameters

Descriptive statistics for feed efficiency and growth traits are reported in Table 3.1. Growth curve parameters of male KR chickens used to be close to the mean values of L (0.14 d-1), K (0.03 d-1), and TI (48.9 d) reported by N'Dri et al. (2007) on male and female chickens issued from a commercial meat-type line used for Label Rouge (LR) production. This indicated that the two lines had similar shape of growth curve. However, while BW1 only 4.9% lower in KR than in Label Rouge, the difference increased with age, up to 19.0% at 10 weeks. By contrast, growth performances of KR chickens were higher than the ones obtained on purebred Thai indigenous Leung Hang khao chicken (Molee et al., 2018). This can be related to differences in the genetic background but also in the feeding and breeding conditions of birds.

A strong positive phenotypic correlation (0.95, $p<0.0001$) was observed between the initial specific growth rate (L) and the maturation rate (K). By contrast, birds with higher specific initial growth rate were characterized by lower age at inflection ($r=-0.85$, $p<0.0001$). These trends were consistent with the genetic correlations reported between growth curves parameters by N'Dri et al. (2007) on LR chickens.

Phenotypic correlations between body weight or feed efficiency measurements and growth curves parameters are reported in Table 3.2. As expected, strong positive phenotypic correlations were observed between L and K parameters on the one hand and early body weight on the other hand. Moreover, a quicker initial growth rate (associated to a higher maturation rate) led to higher body weight all along the rearing period as indicated by marked positive correlations between L (or K) parameter and BW from 1 week to 10 weeks.

Significant positive correlations were found between L and RFI measurements, even if they declined from week 1 (0.55) to week 10 (0.29). Similar trends were observed with K parameter. This could be explained by higher maintenance costs, and feed consumption, of birds characterized by greater body weight at low age. Although RFI and RES estimates used to be positively correlated (from 0.27 to 0.61 according to the age), faster growth rate at young age appeared favorable regarding FCR since low to moderate negative correlation was found between the latter trait and L parameter (from -0.07 to -0.41 according to the age).

In their study on slow-growing native chickens, Wen et al. (2018) showed that birds with the best FCR values had a higher body weight but a level of feed intake similar to the one observed in the whole population. On the other hand, the best RFI

values were associated with lower body weight and lower feed intake compared to the general population. According to the genetic parameters obtained by N'Dri et al. (2006) on LR chickens, improving FCR would result in modifying the growth curve (by limiting initial growth rate) without impacting body weight at slaughter, while improving RFI should decrease feed consumption because birds will be lighter at slaughter. In the current study, when considering the most efficient birds based on FCR criteria (20% of FCR10-ranked birds), we observed an increase in BW at slaughter by comparison to the general population (1540 vs 1460, P=0.15). By contrast, when considering the most efficient birds based on RFI criteria (20% of RFI10-ranked birds), BW at slaughter was lower than in the general population (1382 vs 1460, P=0.15). In both cases, feed intake calculated over the whole rearing period was reduced even if the decreased was more drastic for RFI criteria (-543g, P<0.001) than for FCR criteria (-345g, P<0.01). This illustrated that feed efficiency remains a complex trait resulting from a balance between feed intake and body weight gain, which may vary according to the genotype and the feeding and breeding conditions.

**Table 3.1** Descriptive statistics for feed efficiency and growth parameters.

| Traits | Number | Mean | SD | Maximum | Minimum |
|---|---|---|---|---|---|
| FCR1 | 68 | 1.80 | 0.41 | 3.11 | 1.21 |
| FCR2 | 74 | 1.80 | 0.31 | 2.89 | 1.17 |
| FCR3 | 71 | 1.67 | 0.19 | 2.16 | 1.33 |
| FCR4 | 74 | 1.79 | 0.25 | 2.69 | 1.38 |
| FCR5 | 74 | 1.94 | 0.26 | 2.73 | 1.42 |
| FCR6 | 74 | 2.02 | 0.27 | 2.72 | 1.44 |
| FCR7 | 74 | 2.07 | 0.28 | 1.47 | 2.73 |
| FCR8 | 75 | 2.23 | 0.29 | 3.01 | 1.57 |
| FCR9 | 75 | 2.44 | 0.33 | 3.28 | 1.67 |
| FCR10 | 75 | 2.62 | 0.35 | 3.36 | 1.83 |
| RFI1 (g) | 74 | -1.36 | 25.94 | 49.89 | -62.89 |
| RFI2 (g) | 75 | -0.00 | 46.54 | 91.12 | -103.49 |
| RFI3 (g) | 75 | 0.00 | 65.11 | 187.56 | -169.94 |
| RFI4 (g) | 75 | -0.00 | 90.40 | 228.57 | -198.89 |
| RFI5 (g) | 75 | -0.00 | 124.41 | 327.06 | -273.17 |
| RFI6 (g) | 74 | -7.72 | 169.14 | 338.44 | -393.31 |
| RFI7 (g) | 73 | -3.55 | 241.24 | 608.43 | -453.87 |
| RFI8 (g) | 73 | -3.66 | 295.63 | 731.64 | -580.50 |

**Table 3.1** Continues.

| Traits | Number | Mean | SD | Maximum | Minimum |
|---|---|---|---|---|---|
| RFI9 (g) | 73 | -1.76 | 341.24 | 776.29 | -754.71 |
| RFI10 (g) | 73 | -1.62 | 411.44 | 843.86 | -881.43 |
| BW1 (g) | 72 | 85.06 | 13.77 | 110.00 | 56.00 |
| BW2 (g) | 73 | 169.63 | 23.87 | 220.00 | 115.00 |
| BW3 (g) | 74 | 291.38 | 39.30 | 375.00 | 195.00 |
| BW4 (g) | 74 | 438.51 | 54.20 | 565.00 | 310.00 |
| BW5 (g) | 74 | 590.59 | 67.37 | 750.00 | 460.00 |
| BW6 (g) | 74 | 778.81 | 86.63 | 1,000.00 | 600.00 |
| BW7 (g) | 74 | 997.84 | 107.35 | 1,260.00 | 780.00 |
| BW8 (g) | 74 | 1,155.00 | 128.41 | 1,480.00 | 920.00 |
| BW9 (g) | 75 | 1,283.00 | 167.66 | 1,740.00 | 940.00 |
| BW10 (g) | 75 | 1,460.00 | 193.93 | 1,920.00 | 1,080.00 |
| L ($d^{-1}$) | 75 | 0.12 | 0.01 | 0.15 | 0.08 |
| K ($d^{-1}$) | 75 | 0.03 | 0.00 | 0.04 | 0.02 |
| TI (d) | 72 | 47.04 | 6.27 | 67.02 | 33.44 |

[1]FCR1 to FCR 10 = feed conversion ratio from 1 week to 10 weeks of age; RFI1 to RFI10 = residual feed intake from 1 week to 10 weeks of age; BW1 to BW10 = body weight from 1 week to 10 weeks of age; L = initial specific growth rate; K = maturation rate; TI = age at inflexion

**Table 3.2** Phenotypic correlations between growth curve parameters and feed efficiency or body weight traits.

| **Traits**[1] | **FCR1** | **FCR2** | **FCR3** | **FCR4** | **FCR5** | **FCR6** | **FCR7** | **FCR8** | **FCR9** | **FCR10** |
|---|---|---|---|---|---|---|---|---|---|---|
| L | -0.07 | -0.22 | -0.31* | -0.38* | -0.47** | -0.41* | -0.28* | -0.36* | -0.27* | -0.41* |
| K | -0.12 | -0.27* | -0.31* | -0.37* | -0.43* | -0.36* | -0.25* | -0.33* | -0.19 | -0.34* |
| TI | -0.04 | 0.19 | 0.21 | 0.37* | 0.42* | 0.37* | 0.26* | 0.26* | 0.14 | 0.28* |
| Traits | RFI1 | RFI2 | RFI3 | RFI4 | RFI5 | RFI6 | RFI7 | RFI8 | RFI9 | RFI10 |
| L | 0.55** | 0.63** | 0.52** | 0.47** | 0.26* | 0.26* | 0.26* | 0.29* | 0.30* | 0.29* |
| K | 0.54** | 0.60** | 0.51** | 0.42* | 0.21 | 0.22 | 0.23 | 0.24* | 0.24* | 0.22 |
| TI | -0.58** | -0.49** | -0.39* | -0.27* | -0.06 | -0.08 | -0.11 | -0.14 | -0.14 | -0.14 |
| Traits | BW1 | BW2 | BW3 | BW4 | BW5 | BW6 | BW7 | BW8 | BW9 | BW10 |
| L | 0.70** | 0.79** | 0.82** | 0.82** | 0.77** | 0.73** | 0.64** | 0.62** | 0.54** | 0.65** |
| K | 0.73** | 0.81** | 0.81** | 0.77** | 0.68** | 0.62** | 0.50** | 0.46** | 0.36* | 0.49** |
| TI | -0.66** | -0.77** | -0.72** | -0.66** | -0.55** | 0.50 | -0.37* | -0.35* | -0.20 | -0.35* |

*P < 0.05, ** P < 0.001

[1]FCR1 to FCR 10 = feed conversion ratio from 1 week to 10 weeks of age; RFI1 to RFI10 = residual feed intake from 1 week to 10 weeks of age; BW1 to BW10 = body weight from 1 week to 10 weeks of age; L = initial specific growth rate; K = maturation rate; TI = age at inflexion

### 3.4.2 Phenotypic correlations between meat qualities in breast and thigh meat

Descriptive statistics for meat quality traits are reported in Table 3.3. Breast meat from male KR chickens exhibited low ultimate pH ($pH_u$) with an average value (i.e. 5.58) which could be qualified as 'acid meat' ($pH_u$<5,7). It appeared lower than in previous studies on male slow-growing chickens (from 5.66 to 5.81 in Berri et al., 2005a, Chabault et al., 2012, Muth and Valle Zárate, 2017). Mean $pH_u$ in thigh was higher (i.e. 6.20) and close to previously reported values (6.15 in Berri et al.,

2005a). Literature brings consistent indications that selection for higher body weight and muscle development has been associated with increased breast meat $pH_u$, due to lower glycogen content in breast muscle of fast-growth and high-yield birds (Petracci et al., 2017). Although it was not measured in the current study, the low meat $pH_u$ of KR chickens is consistent with a high glycogen reserve in the breast muscle of this slow-growing line.

**Table 3.3** Descriptive statistics for ultimate pH (pHu), water-holding capacity (WHC), and drip loss (DL) in breast and thigh meat of KR.

| Traits | Number | Mean | SD | Maximum | Minimum |
|---|---|---|---|---|---|
| Breast | | | | | |
| $pH_u$ | 75 | 5.58 | 0.25 | 6.37 | 4.84 |
| WHC (%) | 75 | 78.03 | 5.10 | 91.08 | 64.30 |
| DL (%) | 74 | 8.66 | 3.16 | 18.57 | 0.48 |
| Thigh | | | | | |
| $pH_u$ | 75 | 6.20 | 0.30 | 6.87 | 5.64 |
| WHC (%) | 74 | 82.34 | 4.56 | 92.24 | 74.15 |
| DL (%) | 72 | 9.49 | 3.82 | 21.26 | 1.98 |

$pH_u$ = ultimate pH, WHC = Water Holding Capacity, DL = Drip loss

Post-mortem pH fall is known to be a determining factor of water-holding capacity of poultry meat. The further meat pH is above the isoelectric point of muscle proteins, the stronger the protein charge and the greater the WHC. Thus, minor changes in fresh meat pH can lead to large changes in WHC (Bowker, 2017). Acidic

meat or PSE-like meat are characterized by a poor WHC and negative correlations are usually reported between breast meat $pH_u$ and drip loss, including in slow-growing chickens (Berri et al., 2005). Surprisingly, we did not observe any significant correlation between pHu and WHC or DL in breast; a slight positive correlation (0.26) was observed with WHC in thigh (Table 3.4). This can suggest that other postmortem factors than post-mortem pH decline or antemortem factors have contributed to the variation of the water-holding capacity of KR meat.

**Table 3.4** Phenotypic correlations between ultimate pH (pHu), water-holding capacity (WHC), and drip loss (DL) in breast (above the diagonal) or in thigh (below the diagonal) meat of KR.

| **Traits** | **Coefficient correlation** | | |
|---|---|---|---|
| | **$pH_u$** | **WHC** | **DL** |
| $pH_u$ | - | -0.06 | 0.21 |
| WHC | 0.26* | - | -0.10 |
| DL | 0.06 | -0.24* | - |

*P < 0.05, $pH_u$ = ultimate pH, WHC = Water Holding Capacity, DL = Drip loss

### 3.4.3 Phenotypic correlations between growth or feed efficiency traits and meat quality parameters

Regardless of age, BW was positively correlated with breast meat $pH_u$ (with values ranging from 0.24 to 0.48) (Table 3.5). Correlations with WHC in breast used to be negative (between -0.13 and -0.28) and positive with DL (between 0.08 and 0.29). In accordance with the correlations with BW at young age, $pH_u$ of breast meat

appeared to be significantly positively correlated with initial specific growth rate (0.34) and negatively with age at inflection (-0.35). DL of breast meat was also significantly positively correlated with initial specific growth rate (0.30) and negatively with age at inflection (-0.26). In the case of thigh, while no significant correlation was found with $pH_u$, BW measurements were negatively correlated with WHC (from -0.17 to -0.37) and positively with DL (from 0.07 to 0.30). Maturation rate (K) was negatively correlated with WHC (-0.35) and positively with DL (0.27). A moderate but significant positive correlation (0.24) was found between age at inflection and WHC of thigh meat. Altogether, these results suggested that chickens with higher initial growth rate and maturation rate were characterized by a lower water-holding capacity of the meat. This did not seem to be associated with a propensity to develop more acid meat, since a higher speed of growth (especially in the first weeks) was positively associated with $pH_u$, at least in breast meat.

As shown in Table 3.6, low to moderate levels of correlation were observed between FCR and meat quality traits. They were in favor of a higher $pH_u$ but a lower water retention of breast meat for the most efficient birds. Thus, FCR calculated over the all rearing period (FCR10) was negatively correlated with breast meat $pH_u$ (-0.25) and DL (-0.34) but positively correlated with WHC (0.37). Similar trends were observed for thigh meat, with unfavorable correlations observed with WHC (0.30) and DL (-0.29) and a low correlation with $pH_u$ (0.13). Correlations between Residual Feed Intake (RFI) and meat quality traits used to be low. Thus, no significant correlation was established between RFI after 10 weeks and any of the meat quality measurement, in breast or in thigh.

Several phenotypic and genetic analyses have reported an increase in breast meat $pH_u$ in response to selection for higher body weight and breast muscle development. This was accompanied by metabolic and structural modifications such as a muscle fiber hypertrophy and a decrease in the glycogen storage (Berri et al., 2007b; Le Bihan-Duval et al., 2008; Petracci et al., 2017). Further studies would be needed to characterize which have been the muscular modifications underlying the positive relationship observed between BW and pHu in KR chicken. Very few studies have focused on the link between feed efficiency and meat quality in chicken. In their genetic study on fast-growing chickens, Paiva et al. (2018) reported that selection to improve FCR should impair breast meat color, water-retention and tenderness. Part of these effects were likely related to the unfavorable genetic relationship found between FCR and breast meat $pH_u$ (0.33). The results of the current study suggested that improvement of FCR could have a detrimental effect on water-retention in KR chicken too, even if this unfavorable relationship did not seem mediated by a decrease of meat $pH_u$. On the other hand, relationships between RFI and meat quality traits appeared to be weak, suggesting that the latter trait could be an alternatively criteria of interest in the perspective of a selection for a balance between feed efficiency and meat quality.

**Table 3.5** Phenotypic correlations between body weight or growth curve parameters and meat quality in breast and thigh.

| **Traits**[1] | **Breast** | | | **Thigh** | | |
|---|---|---|---|---|---|---|
| | $pH_u$ | WHC | DL | $pH_u$ | WHC | DL |
| BW1 | 0.42* | -0.28* | 0.29* | -0.16 | -0.37* | 0.30* |
| BW2 | 0.48** | -0.22 | 0.27* | -0.18 | -0.30* | 0.30* |
| BW3 | 0.41* | -0.22 | 0.25* | -0.18 | -0.32 | 0.20 |
| BW4 | 0.41* | -0.19 | 0.23 | -0.27 | -0.26* | 0.25* |
| BW5 | 0.34* | -0.24* | 0.16 | -0.12 | -0.29* | 0.21 |
| BW6 | 0.29* | -0.18 | 0.14 | -0.13 | -0.26* | 0.19 |
| BW7 | 0.24* | -0.13 | 0.08 | -0.06 | -0.23* | 0.14 |
| BW8 | 0.28* | -0.16 | 0.08 | 0.07 | -0.29* | 0.12 |
| BW9 | 0.26* | -0.16 | 0.13 | -0.01 | -0.17 | 0.07 |
| BW10 | 0.41* | -0.28* | 0.19 | -0.07 | -0.35* | 0.23 |
| L | 0.34* | -0.20 | 0.30* | -0.11 | -0.14 | 0.21 |
| K | 0.33 | -0.17 | 0.31 | -0.14 | -0.35* | 0.27* |
| TI | -0.35* | 0.12 | -0.26* | 0.21 | 0.24* | -0.19 |

*P < 0.05, ** P < 0.001, [1]BW1 to BW10 = Body weight from 1 week to 10 weeks; L = initial specific growth rate; K = maturation rate; TI = age at inflexion; pHu = ultimate pH; WHC = water-holding capacity; DL = drip loss

**Table 3.6** Phenotypic correlations between body weight, feed efficiency, growth curve and meat quality in breast and thigh.

| Traits | Breast | | | Thigh | | |
|---|---|---|---|---|---|---|
| | **$pH_u$** | **WHC** | **DL** | **$pH_u$** | **WHC** | **DL** |
| BW10 | 0.41* | -0.28* | 0.19 | -0.07 | -0.35* | 0.23 |
| FCR10 | -0.25* | 0.37* | -0.34* | 0.13 | 0.30 | -0.29* |
| RFI10 | 0.23 | 0.05 | -0.14 | 0.10 | -0.02 | -0.02 |
| L | 0.34* | -0.20 | 0.30* | -0.11 | -0.14 | 0.21 |
| K | 0.33 | -0.17 | 0.31 | -0.14 | -0.35* | 0.27* |
| TI | -0.35* | 0.12 | -0.26* | 0.21 | 0.24* | -0.19 |

BW10 = Body weight at 10 weeks ; FCR10 = Feed conversion ratio at 10 weeks ; RFI10 = Residual feed intake at 10 weeks ; L = initail specific growth rate ; K = maturation rate ; TI = age at inflexion ; $pH_u$ = ultimate pH ; WHC = Water Holding Capacity ; DL = drip loss ; L = initail specific growth rate ; K = maturation rate ; TI = age at inflexion

## 3.5 Conclusions

This study provided new information on the phenotypic relationships between growth performances, feed efficiency and meat quality in slow-growing chicken. According to these first results, the initial growth rate appeared as a determining factor of feed efficiency of KR chicken. Strategy based on improving BW and FCR was likely to impair breast and thigh meat quality, while residual feed intake could constitute an alternatively criteria of interest. The genetic parameters of feed

efficiency and meat quality have now to be investigated in order to elaborate a strategy of selection leading to sustainable KR production.

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# CHAPTER IV

# CHANGING FEED EFFICIENCY AFFECTS ON SECONDARY STRUCTURE PROTEIN AND FLOVOR PRECURSOR IN KR SLOW-GROWING CHICKEN

## 4.1 Abstract

The aim of this study was to investigate the correlation between feed efficiency and flavor precursor, biochemical compound in thigh muscle using 75 male KR chickens. The individual body weight and feed intake of male slow-growing KR chickens were recorded weekly from age of 1 week to 10 weeks for feed conversion ratio (FCR) and residual feed intake (RFI) calculation. At 10 weeks of age, a total of 75 of male KR chicken were slaughteredde-feathering, washed with clean water. Thigh muscle (10 g) were vacuum packed in plastic bags and put in a chilled room at 4°C for 24 h before nucleotides content and Fourier Transform Infrared (FTIR) analysis. Based on FCR and RFI at 10 weeks, a group of extreme 12 high birds and a group of extreme 12 low birds were selected foranalysis. The low FCR chicken had higher adenosine monophosphate (AMP) and inosine contents than high FCR whereas flavor precursor in thigh muscle was no difference based on RFI. The ratio of secondary structure protein showed nonsignificant with high feed efficiency and low feed efficiency of both traits for FCR and RFI. FCR was negatively correlated to AMP and inosine while RFI was positively correlated with inosine 5´-monophosphate

(IMP). FCR and RFI could not impact on changing biochemical compound in thigh muscle.

## 4.2 Introduction

Korat (KR) chicken is a slow-growing line which is a crossbred between Thai indigenous Leung Hang khao and SUT (Suranaree University of Technology) synthetic line. Its meat is recognized for good texture and flavor (Katemala et al. 2018). Nowadays, the thighs and drumsticks are requirement by consumers from East Asia, Mexico, India, Russia or Morocco (Willems, 2018). This indicated the opportunity to contribute KR chicken to the Asia market. However, the low feed efficiency in KR chicken is a weak point to compete in market. Feed efficiency of KR chicken can assess by feed conversion ratio (FCR) at 84 days that is 2.94 with 1,790 g of body weight (Hang et al., 2017) while broiler can reach a weight of approximately 1997 g in 42 day with FCR 2.14 (Jaturasitha et al., 2002). Therefore, genetic improvement for feed efficiency is a significant trait to higher power for the KR chicken.

Feed efficiency is evaluated by feed conversion ratio (FCR) and residual feed intake (RFI). FCR is calculated by the ratio of feed intake (FI) and body weight gain (BWG) while RFI using the difference between the actual animal FI and its estimate FI determined by the growth rate and mean BW (Yi et al., 2018). At port-mortem, the degree of breakdown of ribonucleotides, biochemical in meat is of importance for several flavor and nutrients value. The degradation of adenosine 5´-triphosphate (ATP) to adenosine monophosphate (AMP) to inosine 5´-monophosphate (IMP), hypoxanthine and ribose during aging and cookingof meat is determined in the flavor

development in meat (Cambero et al., 2000). IMP is known to be precursor flavor enhancing (umami taste) which the level of IMP and GMP in meat are considered to increase umami taste in meat (Tikk et al., 2006; Heath, 1970). Fourier transform infrared (FT-IR) has been used to predict the meat quality base on vibration of a molecule excited by IR radiation at a specific wavelength range which this method can be revealed lipid (wavenumber 3000-2800 $cm^{-1}$), protein (i.e. alpha helix structure (wavenumber 1665, 1654, 1645 $cm^{-1}$), beta sheet (wavenumber 1635, 1626, 1614 $cm^{-1}$), carbohydrates (wavenumber 1250- 900 $cm^{-1}$) (Grewal et al., 2015; Argyri et al., 2013; Kirschner, et al., 2004). Previous study reported that high percentage of β-sheet structure in meat might be indicated low protein digestibility because low access to gastrointestinal digestive enzymes (Yu, 2005). This might be revealed the biomolecule functional group marker for biochemical compound and the digestibility indicators in meat that is useful to support the KR chicken to be the outstanding qualification of the chicken.

The information involved in correlation between feed efficiency and flavor, biochemical compound is of crucial importance for the direction of breeding plan. Thigh muscle in chicken is composed the muscle fibers type I, type IIA and type IIB which the ratio of them based on breed, sex and age (Jaturasitha et al., 2008). According to Ryu and Kim (2005), Hwang et al. (2010a) indicated that the different nucleotides content between pork based on the ratio of muscle fiber types resulting in different pork flavor. In pig, the muscle fiber type IIB was negatively correlated with pH and drip loss while muscle fiber type IIA showed positively correlated with both of parameters (Ryu and Kim, 2005). The low pH associated to enhance the flavor in beef while the mechanism to explain for this is poorly understood (Pethick et al., 1995).

Additionally, the lower drip loss in muscle had reduced lactase production and glycolytic substrates led to rapid ATP depletion (Maltin et al., 2003). The study of Paiva et al. (2018) indicated that the chicken with low FCR had higher ultimate pH and drip loss in breast than high FCR chicken. To unravel the correlation between feed efficiency and flavor precursor, biochemical compound in thigh muscle. Therefore, the aim of this study was to investigate the correlation between feed efficiency and flavor precursor, biochemical compound in thigh muscle.

## 4.3 Materials and methods

### 4.3.1 Ethics Statement

All procedures used in this study were approved by the Ethics Committee on Animal Use of the Suranaree University of Technology, Nakhon Ratchasima, Thailand, whose document ID is U1-02631-2559.

### 4.3.2 Animal experimental

A total of 75 of male slow-growing KR chickens from three hatch were used. At hatch, birds were sexed, wing-banded, vaccinated against Marek's disease and coccidiosis disease and individually weighted. All KRs were raised in individual cage under the same environmental and nutritional conditions. They were fed ad libitum using starter diet (21% protein), grower diet (19% protein), and finisher diet (17% protein) at 0-3, 4-6, and 7-10 weeks of age, respectively. The individual body weight and feed intake of male slow-growing KR chickens were recorded weekly from age of 1 week to 10 weeks for FCR and RFI calculation. Based on FCR and RFI at 10 weeks, a group of extreme 12 high birds and a group of extreme 12 low birds were selected foranalysis.

### 4.3.3 Nucleotide content analysis

At 10 weeks of age, total of 75 male KR chicken were slaughtered, de-feathering, washed with clean water. Thigh muscle (10 g) were vacuum packed in plastic bags and putin a chilled roomat 4°C for 24 h before analysis.

The nucleotides content in thigh were individually measured according to the method described by Jayasena et al. (2013). Individual thigh muscle (5 g) were mixed with 30 ml of 0.75 M perchloric acid, homogenized for 30 sec and centrifuged at 2,000 x g (Thermo Fisher Scientific, Langenselbold, Germany) at 4°C for 5 min to extract nucleic acid and then filtered through a filter paper (No.1, Whatman International Ltd.). The filtrate (5 ml) was analyzed using HPLC (HP 1260, Agilent Technologies, USA). The peaks of the individual nucleotides were identified using the retention times for standards: hypoxanthine, inosine, inosine-5'-monophosphate (IMP), adenosine-5'-monophosphate (AMP) (Sigma, USA), and the concentration was calculated using the area for each peak.

### 4.3.4 Fourier Transform Infrared (FTIR) spectroscopy

The individual thigh muscle of bird was vacuum packed in plastic bags and put in a chilled room at 4°C for 24 h. After that, 75 individual thighs were chopped, spread on aluminium foil box (3 x 5 cm). The samples were frozen at -80°C for 24 h. After that freeze dried in a freeze-dryer (Alpha 2-4 LSC plus, Osterode am Harz, Germany) operating at -80°C and 1.65Pa for 24 h. The individual thigh freeze dried samples were minced into powder and stored in desiccator at 37°C for FTIR spectrometer analysis. The proximate compositions of nutrients in the thigh between high FCR group and low FCR group were determined using methods of AOAC (1990).

Biochemical profile changing in the meat of samples were collected using the Attenuated Total Reflectance (ATR)-FTIR spectroscopy with single reflection ATR sampling module and coupled with DTGS detector over the measurement range from 4000-400 $cm^{-1}$. The measurements were performed with a spectral resolution of 4 $cm^{-1}$ with 64 scans co-added (Bruker Optics Ltd, Ettlingen, Germany). Spectral acquisition and instrument control were performed using OPUS 7.2 (Bruker Optics Ltd, Ettlingen, Germany) software.

### 4.3.5 Pre-processing of FTIR data

The FTIR raw spectra data consisted of 960 spectra including technical replicates. The data set was reduced to 192 spectra by averaging over technical replicates. Whole raw spectra were converted to the 2nd derivative, using 13 smoothing points and vector normalized to normalize for the effects of differing sample thickness. The 3000-2800 cm-1 and 1800-900 cm-1 intervals were considered. The association band assignment of functional group with biochemical compound showed in Table 4.1

### 4.3.6 Statistical analysis

The significant difference of meat quality parameters (nucleotides content, biochemical compound) between extreme high and low FCR were carried out using student t- test, and the level of significant was defined as 0.05.

Pearson correlations between FCR, RFI, BW at the different ages, and parameters of growth curve and meat quality traits were calculated using the SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, United States).

**Table 4.1** Association band assignment of functional group with biochemical compound.

| Wave number (cm-1) | Molecular vibrations of functional groups | Functional groups marker | biomolecule contributor |
|---|---|---|---|
| 2955 | C-H asymmetric stretching of -CH3 | C-H | fatty acids |
| 2930 | C-H asymmetric stretching of >CH2 | C-H | fatty acids |
| 2870 | C-H symmetric stretching of -CH3 | C-H | fatty acids |
| 2850 | C-H symmetric stretching of >CH2 | C-H | fatty acids |
| 1740 | >C=O stretching | C=O | lipid esters, triglyceride |
| 1655 | Amide I | C=O | α-helical structures of proteins |
| 1637 | Amide I | C=O | β-pleated sheet structures of proteins |
| 1550-1520 1480-1575 | Amide II band | C-N, N-H | proteins |
| 1468 | C-H deformation of >CH2 | C-H | lipids proteins |
| 1400 | C=O symmetric stretching of COO- group | C=O | fatty acids |
| 1310-1240 1229-1301 | Amide III band | C-N, N-H | proteins |
| 1200-900 | C-O-C, C-O dominated by ring vibrations | C-O-C, C-O | Carbohydrate/glycogen |

References based on Santos et al. (2015), Davis and Mauer (2010).

## 4.4 Results and discussion

### 4.4.1 Nucleotides content in thigh muscle of high and low feed efficiency chicken

Mean and standard error of nucleotides content between high FCR and low FCR chicken are shown in Table 4.2. IMP content in low FCR and high FCR was non differentially significant. Thigh of low FCR chicken contained higher AMP and inosine content than high FCR chicken. The 5′-ribonucleotides, adenosine monophosphate (AMP), inosine monophosphate (IMP), guanosine monophosphate (GMP) and inosine are important in meat flavor (Tikk et al., 2006). Low FCR chicken was associated with high body weight (Wen et al. 2018). In agreement, Chen et al. (2002) reported body weight was negatively correlated to IMP in musculus peronaeus chicken. This could be hypothesized that the thigh muscle of low FCR chicken has higher IMP degradation than high FCR chicken.

**Table 4.2** Comparison nucleotides content mean between HFCR and LFCR.

| Nucleotides | HFCR ± SE | LFCR ± SE | P-value |
|---|---|---|---|
| GMP (mg/g) | 0.13 ± 0.01 | 0.15 ± 0.01 | 0.27 |
| IMP (mg/g) | 4.61 ± 0.28 | 4.25 ± 0.28 | 0.38 |
| AMP (mg/g) | 0.08 ± 0.01 | 0.11 ± 0.01 | 0.02* |
| Inosine (mg/g) | 0.38 ± 0.04 | 0.50 ± 0.04 | 0.04* |

*P value < 0.05, HFCR = High Feed Conversion Ratio group, LFCR = Low Feed Conversion Ratio group, GMP = Guanosine monophosphate, IMP = *Inosine monophosphate*, AMP = Adenosine monophosphate

The average and standard error of nucleotides content between high RFI and low RFI chicken are shown in Table 4.3. The flavor precursor between the low RFI and high RFI chicken was non differentially significant. This indicated improve feed efficiency by RFI may not impact on flavor precursor.

**Table 4.3** Comparison nucleotides content mean between HRFI and LFRI.

| Nucleotides | HRFI ± SE | LRFI ± SE | P-value |
|---|---|---|---|
| GMP (mg/g) | 0.15 ± 0.01 | 0.15 ± 0.01 | 0.92 |
| IMP (mg/g) | 4.44 ± 0.25 | 3.74 ± 0.25 | 0.06 |
| AMP (mg/g) | 0.10 ± 0.01 | 0.11 ± 0.01 | 0.41 |
| Inosine (mg/g) | 0.44 ± 0.04 | 0.53 ± 0.04 | 0.13 |

*P value < 0.05, HRFI = High Residual Feed Intake group, LFCR = Low Residual Feed Intake group, GMP = Guanosine monophosphate, IMP = Inosine monophosphate, AMP = Adenosine monophosphate

### 4.4.2 FTIR spectra of high extreme feed efficiency and low extreme feed efficiency revealed biochemical compound in thigh muscle chicken

The spectral features of the different extreme FCRand RFI are shown in Figure 4.1 and 4.2. FTIR spectra were investigated the biochemical compound in thigh muscle of different feed efficiency chicken (high FCR vs low FCR, high RFI vs low RFI) in the spectral region from 4,000 to 400 $cm^{-1}$. Extreme FCR chicken group, selected of 44 and 38 FTIR spectra of high FCR and low were analyzed. High RFI and low RFI contained 36 and 35 FTIR spectra for analysis. The fingerprint regions of specific interest in this study shown length from 3,000 to 900 $cm^{-1}$ which the FTIR

spectra measurements were carried out to explore the molecular characteristics of functional groups of biochemical compounds in the thigh muscle (i.e. fatty acid, proteins, carbohydrate/glycogen).

The results of semi quantitative analysis of the different extreme FCR and RFI using FTIR spectra in term of ratio for the biomolecule contributor are reported in Table 4.4 and 4.5. Regarding FCR, the low FCR chicken had higher ratio of triglyceride (ester bond) but lower ratio of protein (Amide1, Amide2 and Amide3) than high FCR chicken ($P < 0.05$). Results of ratio fat and protein by FTIR were in the same direction with thigh proximate analysis in this study, crude fat and crude protein of low FCR group were 16.22% and 83.17% while HFCR group were 15.59% and 87.89% respectively. In agreement, Mupeta et al. (2000) reported that high FCR chicken (FCR 4.7) and low FCR chicken (FCR 2.5) contained 73.2%, 69.7% crude protein and 34.1%, 47% crude fat in meat respectively but crude protein and crude fat between high FCR and low FCR were nonsignificant. This indicated FTIR technique has highly sensitivity to detect the different of biochemical compound between group than proximate analysis. It might explain by FTIR technique detected the biochemical compound based on molecule vibration (functional group) and nondestructive for sample indicated that the biochemical compound in meat was not destroyed. Also, Wen et al. (2018) reported FCR was positively correlated with intramuscular fat content in breast and thigh slow-growing broiler. In contrast the results for RFI, the low RFI chicken had higher ratio of fatty acid and triglyceride (fatty acids, ester bond) but lower ratio of protein (Amide1, Amide2) in thigh muscle.

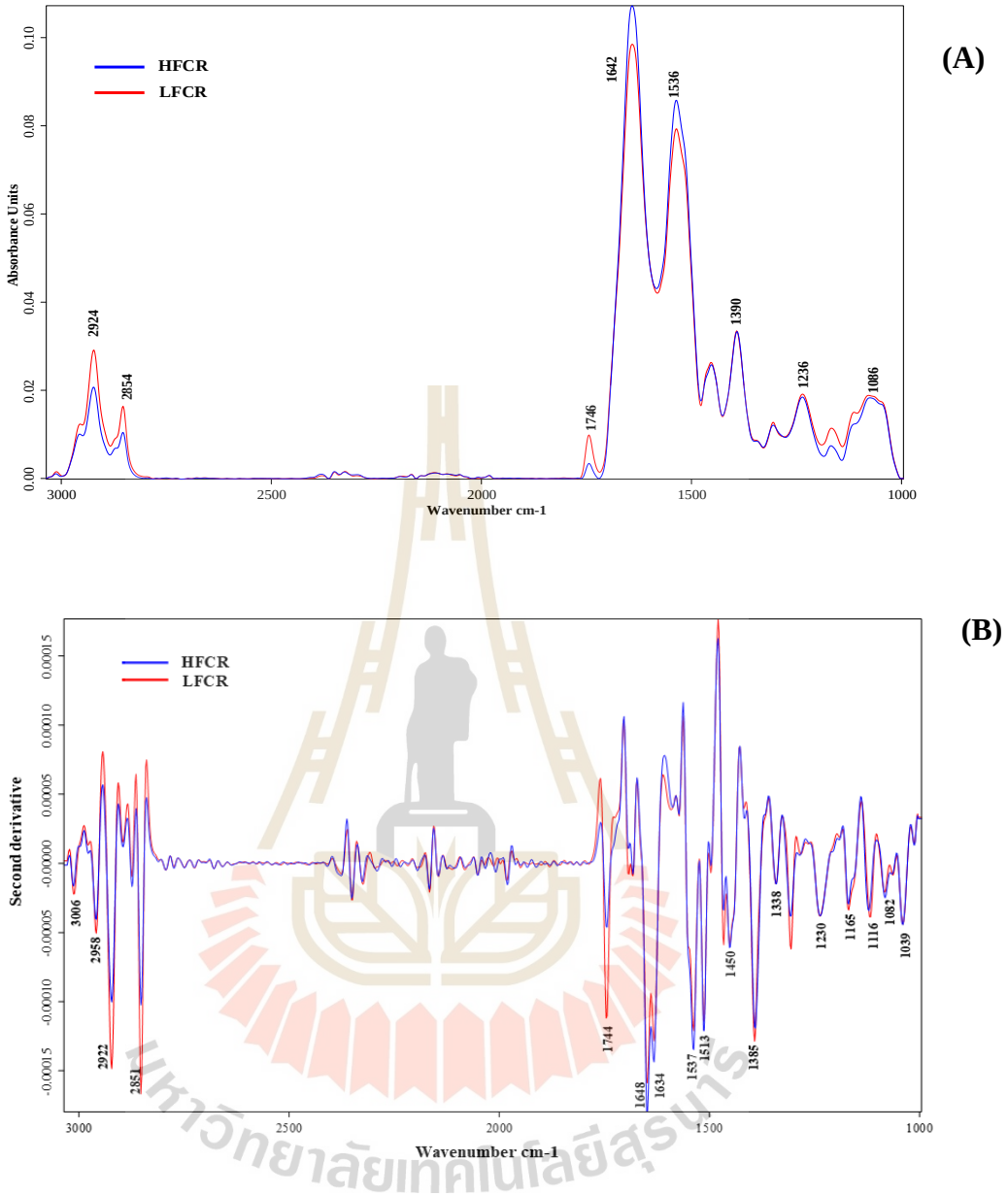


**Figure 4.1 (A)** Average original spectra between high FCR and low FCR in thigh muscle. **(B)** 2nd derivative spectra between high FCR and low FCR. The IR spectra detected in spectra region from 4,000 to 400 $cm^{-1}$, resolution 4 $cm^{-1}$, with 64 scans.

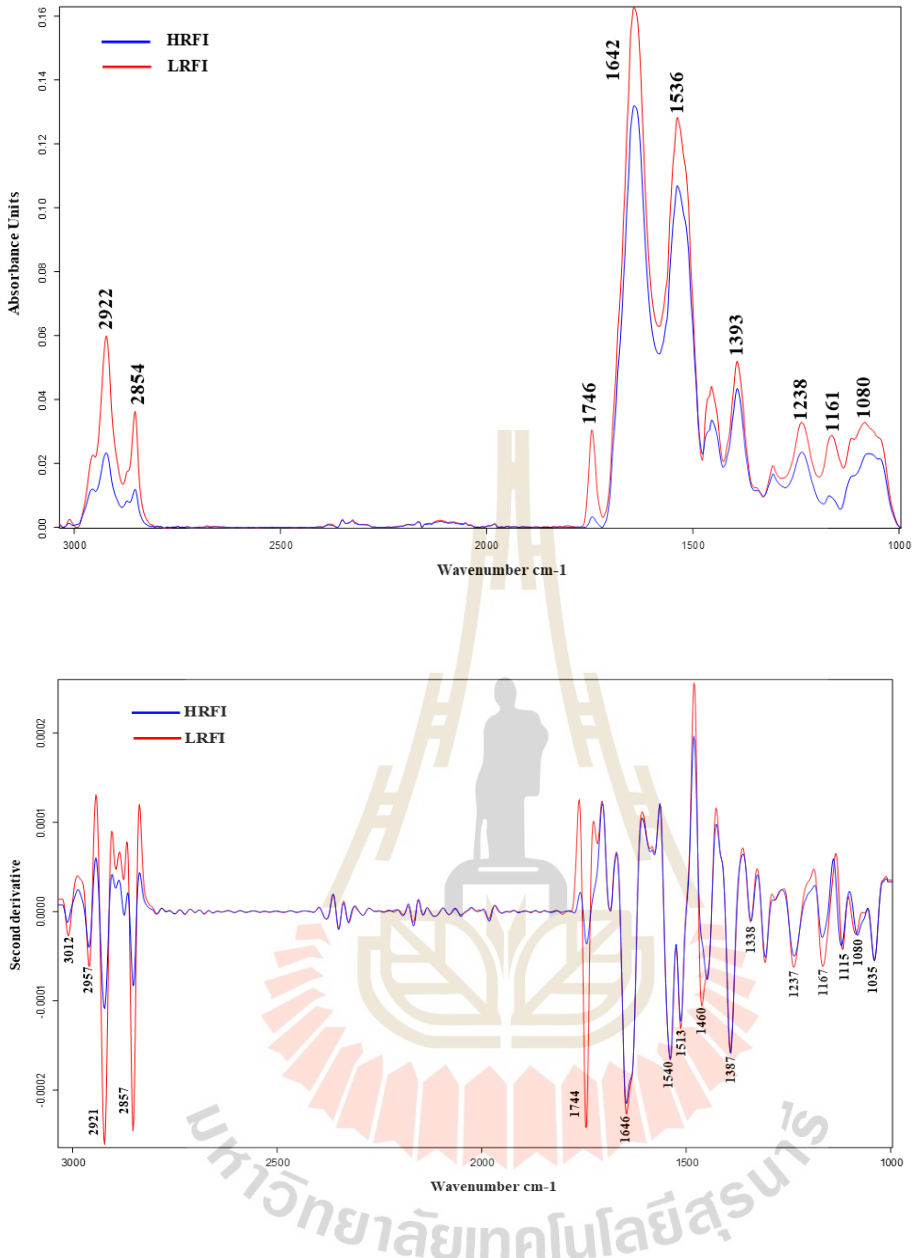


**Figure 4.2** **(A)** Average original spectra between high RFI and low RFI in thigh muscle. **(B)** 2nd derivative spectra between high RFI and low RFI. The IR spectra detected in spectra region from 4,000 to 400 $cm^{-1}$, resolution 4 $cm^{-1}$, with 64 scans.

Based on improving BW and FCR, the different composition of muscle type I, IIA, IIB in thigh was varied by body weight, breed (Jaturasitha et al., 2008). Improving feed intake (FI) and RFI was also associated to fiber area composition, proportion of fiber type II (Zadinová et al., 2019). In addition, the muscle fiber type I and type IIA were positively correlated with umami and richness whereas negatively correlated with muscle fiber type IIB in pork (Hwang et al., 2018b). This might explain by the different ratio of muscle fibers type in thigh of FCR group and RFI group resulting in different biochemical compound. However, the fatty acid profile could be investigated in the further study to explain the ratio of saturated and unsaturated fat between high RFI and low RFI.

**Table 4.4** Comparison percentage of integral area mean between HFCR and LFCR.

| **Functional group (wavenumber)** | **HFCR ± SE** | **LFCR ± SE** | **P-value** |
|---|---|---|---|
| | **% integral area** | **% integral area** | |
| Fatty acids (2800-3000 $cm^{-1}$) | 20.33 ± 0.59 | 23.57 ± 0.59 | 0.06 |
| Ester bond (1740 $cm^{-1}$) | 6.41 ± 0.16 | 7.60 ± 0.16 | 0.04* |
| Amide 1 (1700-1600 $cm^{-1}$) | 24.51 ± 0.72 | 21.06 ± 0.72 | 0.03* |
| Amide 2 (1600-1500 $cm^{-1}$) | 19.66 ± 0.47 | 17.45 ± 0.47 | 0.03* |
| CH bending (1450, 1380 $cm^{-1}$) | 14.25 ± 0.98 | 20.09 ± 0.98 | 0.05 |
| Amide 3 (1338 $cm^{-1}$) | 0.69 ± 0.00 | 0.71 ± 0.00 | 0.02* |
| Carbohydrate/glycogen (1250-900 $cm^{-1}$) | 15.23 ± 0.45 | 15.12 ± 0.55 | 0.89 |

*P value < 0.05, HFCR = High Feed Conversion Ratio group, LFCR = Low Feed Conversion Ratio group

**Table 4.5** Comparison percentage of integral area mean between HRFI and LRFI.

| Functional group (wavenumber) | HRFI ± SE | LRFI ± SE | P-value |
|---|---|---|---|
| | % integral area | % integral area | |
| Fatty acids (2800-3000 $cm^{-1}$) | 16.58 ± 0.65 | 25.55 ± 0.65 | 0.001** |
| Ester bond (1740 $cm^{-1}$) | 2.28 ± 1.06 | 10.89 ± 1.06 | 0.005* |
| Amide 1 (1700-1600 $cm^{-1}$) | 27.01 ± 0.89 | 18.96 ± 0.89 | 0.003* |
| Amide 2 (1600-1500 $cm^{-1}$) | 21.60 ± 1.19 | 16.60 ± 1.19 | 0.04* |
| CH bending (1450, 1380 $cm^{-1}$) | 16.86 ± 1.49 | 14.34 ± 1.49 | 0.30 |
| Amide 3 (1338 $cm^{-1}$) | 0.50 ± 0.07 | 0.55 ± 0.35 | 0.68 |
| Carbohydrate/glycogen (1250-900 $cm^{-1}$) | 15.17 ± 0.77 | 13.13 ± 0.77 | 0.13 |

*P value < 0.05

### 4.4.3 Correlation between secondary structure protein and nucleotides content in thigh muscle

The curve fitting of Amide1 (wavenumber 1700-1600 $cm^{-1}$) to assess the secondary structure protein include alpha helix, beta sheet, beta turn and antiparallel for FCR and RFI are shown in Figure 4.3 and 4.4. Individual thigh of both traits for extremely FCR and RFI were calculated the ratio of alpha helix, beta sheet, beta turn and antiparallel by curve fitting. The results of ratio secondary structure protein between high feed efficiency and low feed efficiency showed in Table 4.6 and 4.7. The ratio of secondary structure protein in thigh muscle showed nonsignificant with high feed efficiency and low feed efficiency of both traits for FCR and RFI.

The correlations between the ratio of secondary structure protein from extreme FCR, RFI and flavor precursor are presented in Table 4.8 and 4.9. Based on FCR, the ratio of secondary structure protein was nonsignificant with flavor precursor

(nucleotides content). The alpha helix from RFI was positively correlated with inosine while antiparallel was negatively correlated to GMP, AMP and inosine. According to Hwang et al. (2018b) reported that inosine was positively correlated with astringency but negatively correlated with richness in pork. Additionally, the higher of beta sheet, beta turn and random coils resulted to hardness in meat (Xu et al., 2011; Herrero, 2008). This indicated the ratio of secondary structure protein could impact on flavor and texture in meat. Interestingly, the ratio of alpha helix, beta sheet, beta turn and antiparallel in Amide1 region of FCR were distinct from RFI. According to Bai et al. (2016) reported the alpha helix structure was positively with in vitro digestibility and protein solubility whereas beta sheet structure was negatively correlated with in vitro digestibility and protein solubility. This may indicate that improving feed efficiency by RFI could get high protein digestibility.

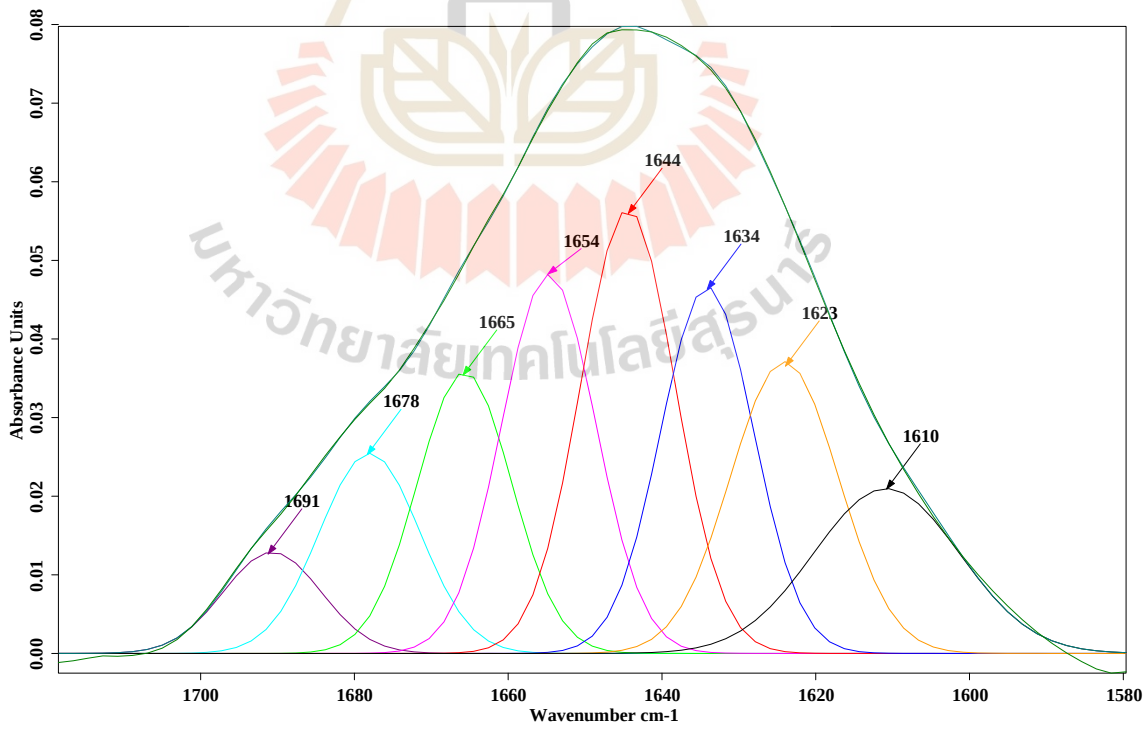


**Figure 4.3** The curve fitting of Amide I and secondary structure protein band assignment in thigh muscle by FCR.

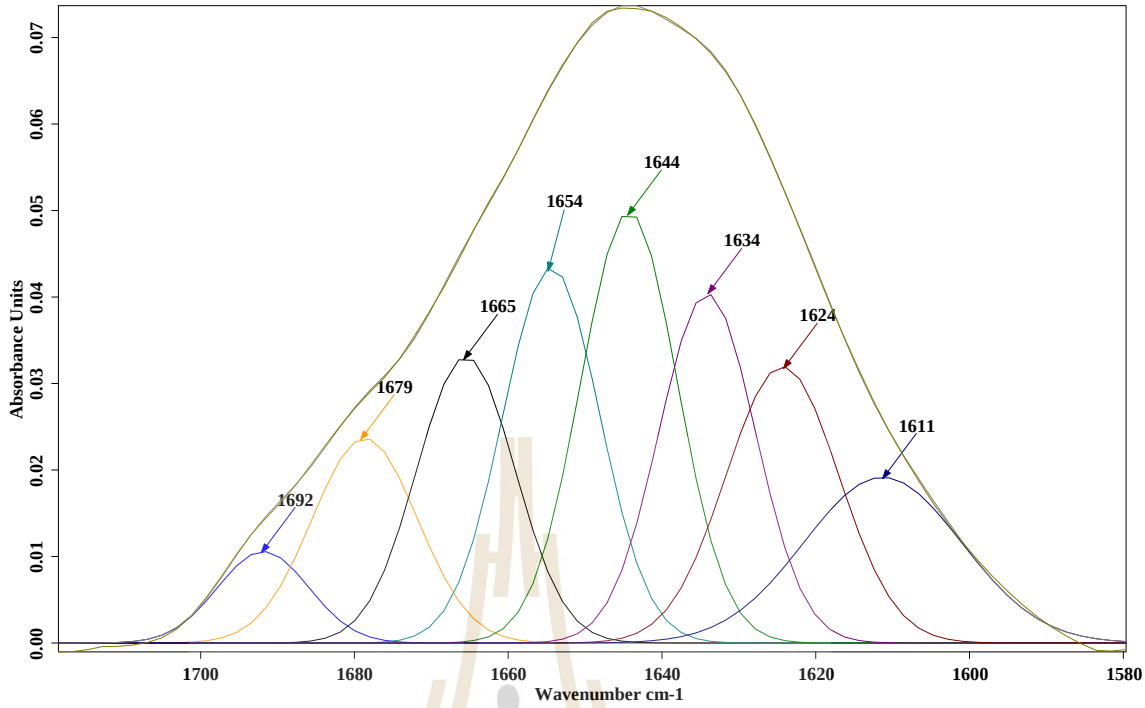


**Figure 4.4** The curve fitting of Amide I and secondary structure protein band assignment in thigh muscle by RFI.

**Table 4.6** Comparison percentage of curve fit mean between HFCR and LFCR.

| Secondary Protein structure (wavenumber) | HFCR ± SE % curve fit | LFCR ± SE % curve fit | P-value |
|---|---|---|---|
| Beta sheet (1610, 1623, 1634 $cm^{-1}$) | 40.45± 0.98 | 41.54± 0.98 | 0.48 |
| Alpha helix (1644, 1654, 1665 $cm^{-1}$) | 45.54 ± 0.71 | 44.25± 0.71 | 0.27 |
| Beta turn (1678 $cm^{-1}$) | 9.74 ± 0.75 | 10.13± 0.75 | 0.73 |
| Antiparallel (1691 $cm^{-1}$) | 4.27± 0.60 | 4.07± 0.60 | 0.82 |

*P value < 0.05, HFCR = High Feed Conversion Ratio group, LFCR = Low Feed Conversion Ratio group

**Table 4.7** Comparison percentage of curve fit mean between HRFI and LRFI.

| Secondary Protein structure (wavenumber) | HRFI ± SE % curve fit | LRFI ± SE % curve fit | P-value |
|---|---|---|---|
| Beta sheet (1611, 1624, 1634 $cm^{-1}$) | 35.66 ± 0.54 | 36.03 ± 0.54 | 0.66 |
| Alpha helix (1644, 1654, 1665 $cm^{-1}$) | 51.05 ± 0.60 | 50.77 ± 0.60 | 0.76 |
| Beta turn (1679 $cm^{-1}$) | 9.24 ± 0.28 | 9.14 ± 0.28 | 0.81 |
| Antiparallel (1692 $cm^{-1}$) | 4.05 ± 0.18 | 4.06 ± 0.18 | 0.97 |

*P value < 0.05

**Table 4.8** Correlation of secondary structure protein from FCR and nucleotides content in thigh.

| Traits | GMP | IMP | AMP | Inosine |
|---|---|---|---|---|
| Beta sheet (1610, 1623, 1634 $cm^{-1}$) | -0.03 | -0.11 | 0.28 | -0.11 |
| Alpha helix (1644, 1654, 1665 $cm^{-1}$) | 0.04 | 0.10 | -0.38 | -0.13 |
| Beta turn (1678 $cm^{-1}$) | 0.27 | 0.04 | 0.19 | 0.27 |
| Antiparallel (1691 $cm^{-1}$) | -0.14 | 0.02 | -0.04 | -0.14 |

*P value < 0.05, GMP = Guanosine monophosphate, IMP = Inosine *monophosphate*, AMP = Adenosine monophosphate

**Table 4.9** Correlation of secondary structure protein from RFI and nucleotides content in thigh.

| Traits | GMP | IMP | AMP | Inosine |
|---|---|---|---|---|
| Beta sheet (1611, 1624, 1634 $cm^{-1}$) | 0.16 | -0.02 | 0.39 | -0.01 |
| Alpha helix (1644, 1654, 1665 $cm^{-1}$) | 0.18 | -0.06 | 0.32 | 0.53** |
| Beta turn (1679 $cm^{-1}$) | 0.03 | -0.06 | -0.40 | -0.05 |
| Antiparallel (1692 $cm^{-1}$) | -0.46* | 0.16 | -0.52* | -0.61** |

*P value < 0.05, **P value < 0.01, GMP = Guanosine monophosphate, IMP = *Inosine monophosphate*, AMP = Adenosine monophosphate

### 4.4.4 Correlation between feed efficiency and nucleotides content, biochemical compound in thigh muscle

The correlation between feed efficiency (FCR, RFI) and meat quality is shown in Table 4.10. The results showed FCR was negatively correlated with AMP and inosine while RFI was positively correlated with IMP. The report of Tikk et al. (2006) reported that IMP and its degradation products are of importance to flavor development in meat. Therefore, the result indicated that improving FCR or RFI may an effect on flavor.

**Table 4.10** Correlation of feed efficiency and meat quality in thigh.

| Traits | FCR | RFI |
|---|---|---|
| GMP | -0.21 | 0.07 |
| IMP | 0.14 | 0.33* |
| AMP | -0.31* | -0.15 |
| Inosine | -0.38* | -0.13 |
| Fatty acids (2800-3000 $cm^{-1}$) | 0.08 | -0.05 |
| Ester bond (1740 $cm^{-1}$) | 0.11 | -0.06 |
| Amide 1 (1700-1600 $cm^{-1}$) | -0.05 | -0.07 |
| Amide 2 (1600-1500 $cm^{-1}$) | 0.15 | 0.04 |
| CH bending (1450, 1380 $cm^{-1}$) | -0.15 | -0.18 |
| Amide 3 (1338 $cm^{-1}$) | -0.23 | -0.20 |
| Carbohydrate/glycogen (1250-900 $cm^{-1}$) | -0.10 | -0.10 |

*P value < 0.05, GMP = Guanosine monophosphate, IMP = Inosine monophosphate, AMP = Adenosine monophosphate



## 4.5 Conclusions

This study explored new information on the correlation between FCR, RFI and nucleotides content (flavor precursor), biochemical compound in thigh muscle slow-growing chicken. Improving FCR or RFI may an effect on flavor in thigh muscle. The ratio of secondary structure protein in thigh muscle may impact on flavor precursor and texture.

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# CHAPTER V

# REVEALING PATHWAYS AND GENES ASSOCIATED WITH FEED EFFICIENCY AND MEAT QUALITY IN KR CHICKEN

## 5.1 Abstract

The objective of this study was to investigate genes and molecular pathways involved in feed efficiency and meat quality in slow-growing KR chicken by using 75 male KR chickens reared up to 10 weeks. Individual body weight and feed consumption were recorded weekly from 1 week to 10 weeks of age. Feed conversion ratio (FCR) and residual feed intake (RFI) were calculated for each week. At 10 weeks of age, chickens were slaughtered and ultimate pH ($pH_u$), water-holding capacity (WHC) and drip loss (DL), biochemical compound by FTIR were collected after 24 h. Based on FCR at 10 weeks, a group of 12 high FCR birds and a group of 9 low FCR birds were selected for transcriptomic analysis of thigh muscle using microarray. No gene was differentially expressed between high FCR and low FCR group. However, correlation analysis using WGCNA package revealed that mitochondrial gene expression, mitochondria respiratory chain complex assembly, mitochondrial translation and positive regulation of mitochondrial translation pathways and the 4 genes (ACD, BIRC5, COA3, MYL9) might be play a crucial role in feed efficiency and meat quality in KR slow-growing thigh muscle.

## 5.2 Introduction

Meat quality of slow-growing chicken becomes to be trend in poultry industry as a result of consumers becoming increasingly concerned about dietary health, flavor and texture (Lee et al., 2017; Aral et al., 2013; Jayasena et al., 2013; Zhao et al., 2011; Ellis et al., 2002). Korat (KR) chicken is a slow-growing line which is a crossbred between Thai indigenous Leung Hang khao and SUT (Suranaree University of Technology) synthetic line. Its meat is recognized for good texture and flavor (Katemala et al. 2018). Feed efficiency that is represented by average Feed conversion ratio (FCR) at 84 days of age is 2.94 with 1,790 g of slaughter body weight (Hang, 2017). This is higher than commercial broiler, and leads to high cost of production which is the significant weak point of this chicken line. Genetic improvement for the trait is therefore necessary. The improvement, however, must not negatively impact the quality of meat that is the outstanding qualification of the chicken.

Considering meat quality, technological quality of the meat, such as ultimate pH, water holding capacity and drip loss are important parameters to explain the post-mortem processing involved with the texture in meat. Flavor enhancing can be investigated by Guanosine monophosphate (GMP) and nucleotide contents including Inosine monophosphate (IMP), Adenosine triphosphate (ATP), Adenosine monophosphate (AMP), Inosine and Hypoxanthine levels (Lee and Lee, 1996). In term of nutrients, biochemical compound (lipid, protein and carbohydrate) in meat are necessary to be investigated. Fourier transform infrared (FTIR) spectroscopy method was applied to detect the meat quality based on vibration of a molecule excited by IR radiation at a specific wavelength range (Kirschner, et al., 2004). This way, the total composition of

the samples can be revealed, including proteins, fatty acids, carbohydrates, nucleic acids, and lipopolysaccharides.

Feed efficiency (i.e., residual feed intake; RFI, feed conversion ratio; FCR) and meat quality are complex traits involving brain-gut axis and hormonal, non-hormonal factors, enteral absorption, type of muscle and structure of muscle fiber (Reyer et al., 2015; Song et al., 2013; Tougan et al., 2013). Previous study reported that FCR was positively correlated with intramuscular fat in breast and thigh slow-growing broiler while RFI was non significantly correlated with meat quality (Wen et al., 2018). Additionally, FCR was negatively correlated to drip loss and lightness in breast broiler (Paiva et al., 2018). This indicated that improving FCR could have an impact on meat quality in chicken. Thus, the pathways and genes involved both traits will deeper understand for breeding plan.

Currently, the whole legs (thighs, drumsticks) are preferred by consumers from East Asia, Mexico, India, Russia or Morocco (Willems, 2018). Based on the literature, broiler with high feed efficiency had mostly up-regulated genes in breast muscle associated with inflammatory response and free radical scavenging (Zhou et al., 2015). It was also reported that 41 genes were significantly differently expressed between high RFI and low RFI in duodenal tissue of layer chicken with genes involved in digestibility, metabolism and energy homeostasis in duodenal tissue (Yi et al., 2015). Moreover, the expression of microRNA (miRNA) in breast of high feed efficiency and low efficiency was differentially expressed and were associated with calcium signaling, axonal guidance signaling, and NRF2-mediated oxidative stress response pathways (Khatri et al., 2018). The genetic information on pathways and genes involved in feed efficiency and flavor, texture, biochemical compound in thigh are still

not explored. Therefore, the aim of this study was to investigate genes and molecular pathways involved in feed efficiency and meat quality in slow-growing KR chicken.

## 5.3 Materials and Methods

### 5.3.1 Ethics Statement and Experimental chickens

This experiment was performed at Suranaree University of Technology, (SUT), Farm. The experimental use of chickens was reviewed and approved by Ethics Committee on Animal Use of the Suranaree University of Technology, Nakhon Ratchasima, Thailand, whose document ID is U1-02631-2559.

Extreme birds for FCR (high FCR from 2.99 to 3.36, low FCR from 1.83 to 2.26) from Korat line of previous generation were used to select sire and dam, and the parents were used to produce 75 male KR chickens. At hatching, chickens were sexed by vent sexing, wing-banded, and vaccinated against Marek's disease. Thereafter, they were vaccinated follow the recommendation of Department of Livestock development, Thailand. Individual cages size 63 cm x 125 cm x 63 cm with floor covered with rice hull, were used to raise chicken individually. They were fed ad libitum using starter diet (21% protein), grower diet (19% protein), and finisher diet (17% protein) at 0-3, 4-6, and 7-10 weeks of age, respectively. The nipple automatic watering system was individually installed in each cage and water was freely available for birds.

### 5.3.2 Data and samples collection

Individual body weight and feed consumption were recorded weekly from 1 week to 10 weeks of age. Feed conversion ratio (FCR) and residual feed intake (RFI) were calculated for each week. Based on FCR at 10 weeks, a group of extreme

12 high FCR birds and a group of extreme 9 low FCR birds were selected for further analysis.

Birds were killed at 10 weeks of age. They were stunned by electricity, bleeded, scalded at 60°C, de-feathered, and carcass was washed and put in chill room (4°C). A piece of thigh muscle was immediately frozen in liquid nitrogen and stored at -80°C before for RNA extraction. The rest of thigh muscle was used to measure parameters of meat qualities.

### 5.3.3 Meat quality measurements

The ultimate pH ($pH_u$) was measured at 24h postmortem by directly inserting the probe into the thigh muscle with a portable pH-meter (pHCore-kit, Satorius Lab Instruments GmbH, Goettingen, Germany) after calibration using buffers (pH 4.01 and 7.00) at room temperature according to recommendations.

Samples that weighed approximately 4-5 g with a size of approximately 1.0 × 2.0 × 0.5 cm (width, length, and height, respectively) were wrapped in absorption pads, placed in polyethylene bags, stored for 24 h at 4°C and weighed again to calculate the percentage of drip loss (DL).

The water-holding capacity (WHC) index of meat samples was determined according to a method based on Sakata, et al. (1993). Individual breast or thigh muscle was chopped and weighed (5 g). Sample were placed on nylon net and wrapped with 3 pieces of filter paper (Whatman No. 4). Wrapped samples were centrifuged at 3000 x g for 20 min (Thermo Fisher Scientific, Langenselbold, Germany) to calculate WHC.

### 5.3.4 Nucleotide content analysis

The nucleotides content in thigh were individually measured according to the method described by Jayasena et al. (2013). Individual thigh muscle (5 g) were mixed with 30 ml of 0.75 M perchloric acid, homogenized for 30 sec and centrifuged at 2,000 x g (Thermo Fisher Scientific, Langenselbold, Germany) at 4°C for 5 min to extract nucleic acid and then filtered through a filter paper (No.1, Whatman International Ltd.). The filtrate (5 ml) was analyzed using HPLC (HP 1260, Agilent Technologies, USA). The inosine-5'-monophosphate (IMP), guanosine monophosphate (GMP), adenosine diphosphate (ADP), adenosine-5'-monophosphate (AMP), hypoxanthine and inosine (Sigma, USA) were used to be standard. The concentration of all were calculated using the area for each peak.

### 5.3.5 Fourier-transform infrared spectroscopy (FTIR) analysis

Thigh samples were chopped and spread on aluminium foil boxes. Samples were frozen at -80°C for 24 h and dehydrated for 24 h in a laboratory freeze-dryer. Freeze-dried thigh samples were grinded into powder.

The infrared spectra were collected using the Attenuated Total Reflectance (ATR)-FTIR spectroscopy with single reflection ATR sampling module and coupled with DTGS detector over the measurement range from 4000-400 cm-1. The measurements were performed with a spectral resolution of 4 cm-1 with 64 scans co-added (Bruker Optics Ltd, Ettlingen, Germany). The peak areas of integration were done using OPUS7.2 software (Bruker Optics Ltd, Ettlingen, Germany). Spectra from each sample group were analyzed by using Principal Component Analysis (PCA). Data were preprocessed by performing second derivative and then normalized using Extended Multiplicative Signal Correction using the spectral regions from 3000-2800

cm-1 and 1800-900 cm-1 using Unscrambler 10.1 software (CAMO, Oslo, Norway). The spectral changes of the functional groups were performed at the integral area from second derivative spectra of each peak such as lipids (3000-2800 cm-1), proteins (1800-1600 cm-1), CH bending (1500-1300 cm-1), amide III (1300-1200 cm-1) and carbohydrates (1200-900 cm-1).

### 5.3.6 Statistical analysis

The significant difference of meat quality parameters between two groups high and low FCR were carried out using student t- test, and the level of significant was defined as 0.05.

### 5.3.7 Isolation of total RNA

Total RNA from thigh muscle of 12 high and 9 low FCR chickens were extracted by TRIzol reagents (Invitrogen, San Diego, CA). Briefly, after mixing with TRIzol, thigh muscle sample was transferred into the 1.5 ml microtube with chloroform and inclubated for 5 min. Samples were centrifugated at 7500 rpm (Thermo Fisher Scientific, Langenselbold, Germany) at 4 deg for 10 min. Pellet was precipitated using Isopropanol and washed with 75% ethanol. Pellet was dried at 25°C for 5 min. RNA pellet was re-suspended in 20 µl Nuclease-free water.

Quality of total RNA was assessed using a NanoDrop ND-1000 spectophotometer (Thermo Fisher Scientific, Walthman, MA, USA). 260/280 and 260/230 ratios of all samples in this study were above 2.0. RNA integrity Number (RIN) was assessed using RNA 6000 Nano chips (Agilent Technologies, Santa Clara, CA, USA). RIN for all samples were above 8.0.

### 5.3.8 Microarray analysis

Microarray analyzes were performed using an 8 x 60K Agilent Technologies array (Palo Alto, CA, USA). that contains 62,976 probes (Platform GPL20588 in the U.S. National Center for Biotechnology Information Gene Expression Omnibus (GEO) microarray database). The steps of labelling, microarray processing, hybridization control, and the other internal controls were performed by the @BRIDGe platform (INRA, UMR GABI, Jouy-en-Josas,). Cyanine-3 (Cy3) labeled cRNA were prepared using 200 ng of total RNA using the One-Color Low Input Quick Amp labeling kit (Agilent Technologies, Santa Clara, CA, USA) following the recommened protocol. For each sample, 600 ng of Cy3-labeled cRNA (specific activity > 6.0 pmol Cy3/µg of cRNA) were fragmented at 60°C for 30 min in a reaction volume of 25 µl containing 25x Agilent Fragmentation buffer and 10x Agilent Blocking Agilent following the manufacturer's instructions. Subsequently, 25 µl of 2x Agilent Hybridization Buffer were added to fragmentation mixture and hybridized to the SuperPrint G3 Custom GE 8 x 60K (Agilent Technology, design ID: G4102A) for 17 h at 65°C in rotating Agilent hybridization oven (Agilent Technologies). The microarray data were submitted to the Gene Expression Omnibus (GEO) microarray database.

External quality control was carried out using the principle component analysis (PCA), and correlation analysis across arrays by Pearson correlation coefficient. Samples and hybridizations were considered of good quality.

### 5.3.9 Reannotation of the microarray chip on the Galgal5 chicken genome

The microarray chip was re-annotated to fetch the chicken genome Galgal5 version. The transcriptome alignment on the Galgal5 chicken assembly

(EntrezGene database) of all the probes deposited on the chip was carried out using the blastn algorithm (hybridization tolerance of two mismatches and a single probe-associated gene as parameters), which is available in the BLAST + suite (ncbi-blast-2.6.0 +). Probes with a hybridization tolerance of two mismatches and a single probe-associated gene were considered correctly annotated and were kept for further analysis

**5.3.10 Differential analysis**

The R/Bioconductor package Limma version 3.36.3 (Linear Models for microarray data) was used to identify probes differentially expressed between high and low FCR. The fluorescence signal of the 41,350 expressed probes were log2 transformed and then normalized by median of each array. The difference of expression between the high FCR and low FCR was tested using a moderated-statistic in the linear model for each of probe. In the context of multiple testing, P-values were adjusted by the Benjamini-Hochberg's method to control the False Discovery Rate (FDR). Differences in expression between high and low FCR were computed as the log2 transformation of the fold-change (lfc). Probes with an adjusted P-value $\leq 0.05$ were considered as differentially expressed between high and low FCR. Then, differentially expressed probes expression were summarized by gene. Top genes were defined by the lowest raw p-value. The scaled expression of the top genes by row was shown in a cluster heat map to find clusters of genes and clusters of samples with a similar profile. A gradient colour from green corresponding to the lowest expression to red corresponding to the highest expression was set in the heat map. Hierarchical clustering on the scaled gene expression for top genes was based on the Pearson correlation distance and a Ward aggregation criterion.

### 5.3.11 Co-expression network analysis

To study the correlations between gene expression levels and quantitative phenotypes as a complementary approach to the differential analysis, we applied a co-expression analysis using the R package Weighted Correlation Network Analysis (WGCNA) (Langfelder and Horvath, 2008).

### 5.3.12 Network construction

The co-expression analysis was performed using an expression matrix based on the 21 samples and 11,829 expressed and annotated genes. The unsigned connected network was built based on the adjacency matrix between genes. From the gene expression matrix, Pearson's correlations between every pair of genes were computed and raised to a selected power of $\beta=9$ using the pickSoftThreshold function to reach a scale-free topology index ($R^2$) of at least 0.80. The adjacency matrix was turned into a Topological Overlap Measure (TOM) matrix, which can be used to assess the degree of shared neighbours between pairs of genes.

### 5.3.13 Module identification

A hierarchical clustering of the genes based on the TOM dissimilarity measure and average link followed by a modular height cut-off value of branches in the hierarchical tree using the cutreeDynamic function (deepSplit = 2, minClusterSize = 30) was performed to detect modules of co-expressed genes. The module eigengene, which was the first principal component of each module and represented the expression value of each module, was calculated. Modules with expression profiles that were very similar were merged (height cut-off of 0.15 in the dendrogram) since there was a high probability that genes belonging to these modules are highly co-expressed.

### 5.3.14 Module-trait relationships

The module eigengene was used to detect biologically relevant modules. Indeed, module-trait relationships were estimated using Pearson's correlation ($P<0.05$) between the module eigengene and each trait of interest (digestive parameters, meat quality parameters). Genes for each module with high Gene Significance ($GS \geq 0.6$) corresponding to the absolute value of the correlation between gene expression and the trait of interest and high Module Membership ($MM \geq 0.7$), which was defined as the absolute value of the correlation of the module eigengene and the gene expression profile, were defined as hub genes.

### 5.3.15 Functional gene ontology enrichment analysis

All expressed genes on the chip were annotated by Gene Ontology (GO) (Ashburner, et al., 2000; The Gene Ontology, 2017) for Biological Process categories according to the NCBI EntrezGene database using orthologues. Functional enrichment tests were performed using the ViSEAGO R package, which is available at https://forgemia.inra.fr/umr-boa/viseago. Enrichment for functions, supported by GO terms, within each modules of genes from WGCNA analysis was tested using a Fisher's exact test and the "classic" algorithm ($P<0.01$) with all expressed genes used as the background. Biological functions were explored using the concept of semantic similarity (Wang's similarity).

## 5.4 Results and discussion

### 5.4.1 Meat quality parameters between high FCR and low FCR birds

The average and standard deviation of meat quality parameters between high FCR and low FCR birds are shown on Table 5.1. The $pH_u$ in thigh muscles of KR

chicken was closed to previously reported values (6.15 in Berri et al., 2005). While, drip loss had higher than indigenous chickens (from 2.53 to 5.06) reported by Jaturasitha et al. (2008).

Thigh muscle from low FCR chicken had higher contents of nucleotide (AMP, Inosine), lipid (CH-bending) but lower protein (Amide1) than high FCR chicken. In agreement, Jayasena, et al. (2013) reported that thigh muscle from Korean native chicken (that exhibit low feed efficiency as well) contained higher crude protein, Inosine but lower crude fat than broiler and also found no difference in pH, WHC and IMP in thigh muscle. Moreover, the study of Yongsawatdigul et al. (2016) reported an increase of Inosine and hypoxanthine indicated a degradation of GMP and IMP contents in meat and they also found high total lipid (based on Raman FTIR) in thigh muscle of broiler that might extend lipid oxidation and protein oxidation causing protein denaturation. This might indicate that improving feed efficiency by controlling FCR could have an effect on faster degradation of IMP and changing nutrients value (low protein, high fat) in thigh muscle.

**Table 5.1** Comparison of meat quality parameters between extreme high FCR and low FCR (mean ± SE) in thigh muscle.

| Parameters | HFCR | LFCR | P-value |
|---|---|---|---|
| Ultimate pH | 6.18 ± 0.08 | 6.06 ± 0.07 | 0.28 |
| WHC (%) | 85.01± 1.74 | 81.10 ± 1.11 | 0.07 |
| DL (%) | 8.22 ± 0.91 | 11.16 ± 1.24 | 0.07 |
| GMP (mg/g) | 0.13 ± 0.01 | 0.15 ± 0.01 | 0.27 |
| IMP (mg/g) | 4.61 ± 0.21 | 4.25 ± 0.33 | 0.38 |
| AMP (mg/g) | 0.08 ± 0.00 | 0.11 ± 0.01 | 0.02* |
| Inosine (mg/g) | 0.38 ± 0.02 | 0.50 ± 0.05 | 0.04* |
| Lipid (%) | 16.20 ± 1.02 | 17.69 ± 1.85 | 0.57 |
| Ester carbonyl of phospholipids (%) | 3.73 ± 0.91 | 4.37 ± 0.93 | 0.64 |
| Amide1 (%) | 25.47 ± 1.02 | 18.61 ± 0.94 | 0.00** |
| Amide 2 (%) | 19.56 ± 0.78 | 19.05 ± 0.84 | 0.65 |
| CH bending (%) | 14.26 ± 0.81 | 17.48 ± 0.86 | 0.02* |
| Amide 3 (%) | 0.52 ± 0.22 | 0.50 ± 0.05 | 0.80 |
| carbohydrate and glycogen (%) | 14.29 ± 0.32 | 14.24 ± 0.48 | 0.93 |

HFCR = High Feed Conversion Ratio, LFCR = Low Feed Conversion Ratio, WHC = Water Holding Capacity, DL = Drip loss, GMP = Guanosine monophosphate, IMP = *Inosine monophosphate,* AMP = Adenosine monophosphate, lipid = wave numbers region 3000-2800 $cm^{-1}$, Ester carbonyl of phospholipids = wave number region 1743 $cm^{-1}$, Amide1 = wave number region 1700-1600 $cm^{-1}$, Amide 2 = wave numbers region 1600-1500 $cm^{-1}$, CH bending = wave numbers region 1450, 1380 $cm^{-1}$, Amide 3 = wave numbers region 1338 $cm^{-1}$, carbohydrate and glycogen = wave numbers region 1250-900 $cm^{-1}$

### 5.4.2 Differential analysis

In order to characterize the thigh muscle transcriptome of high and low FCR Korat chickens, this study used a 60K chicken microarray chip with 62,976 probes. Differential analysis between low and high FCR birds was performed. Nevertheless, no genes was found to be significantly differentially expressed between the two groups (Figure 5.1). It might be due to the high variation within each group. As shown in Figure 5.2, biological replicates from high FCR and low FCR were not grouping together but rather spread over the two first MDS axis. However, heatmap of the top 50 genes based on raw p-value on differential analysis (Figure 5.3) indicated a discrimination between high and low FCR groups.

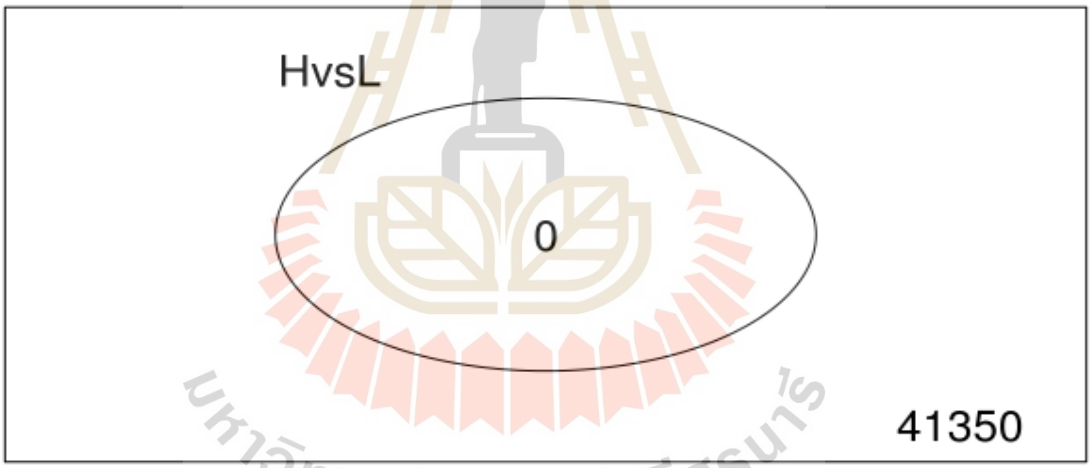


**Figure 5.1** The number of different gene expression from high FCR (H) and low FCR (L).

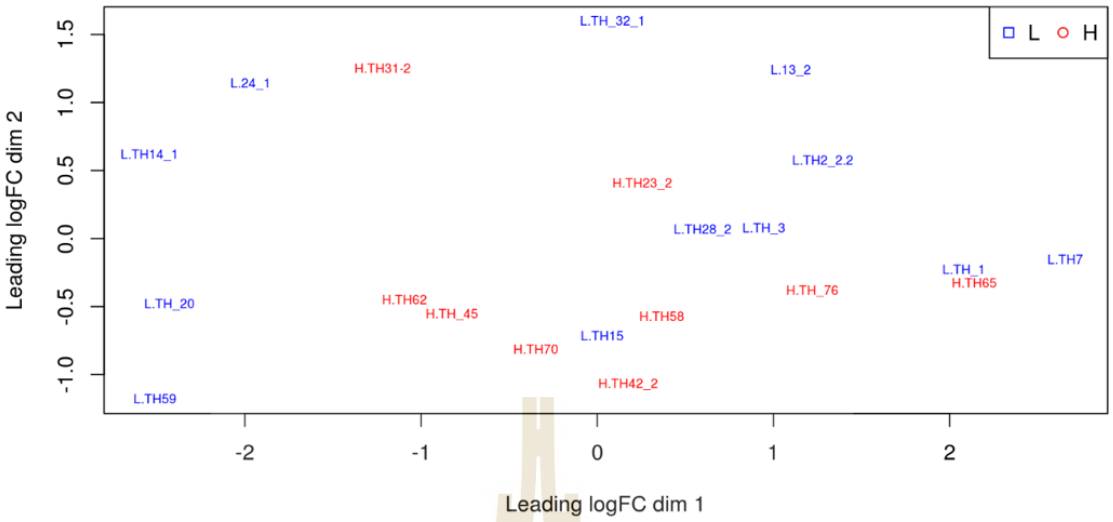


**Figure 5.2** MDS plot of representation biological samples from high FCR (H) and low FCR (L).

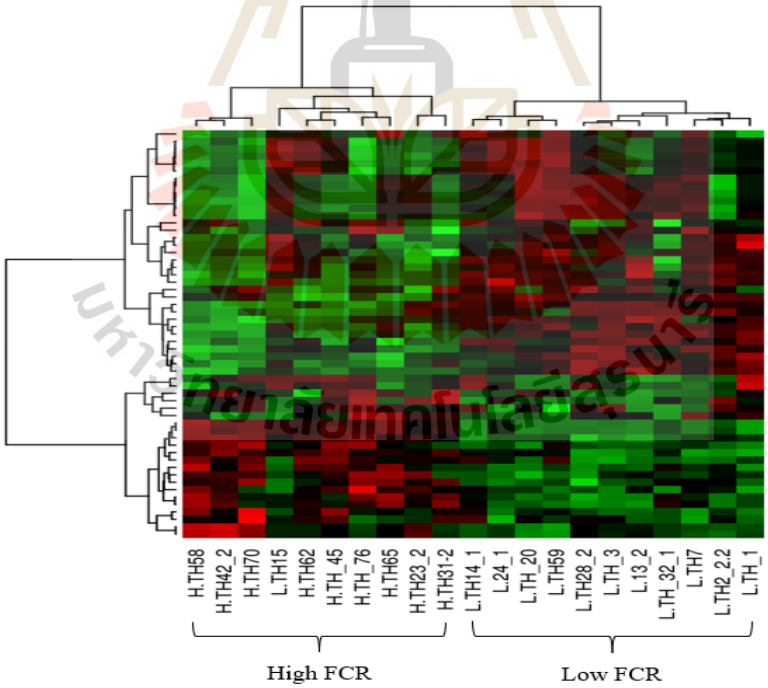


**Figure 5.3** Heat map of top 50 genes (lowest p-values) between high FCR and FCR individuals. Over-expression is represented in red while under-expression is represented in green for each gene.

### 5.4.3 Weighted gene co-expression network analysis and module identification

WGCNA package was used to identify co-expression gene modules and correlate them to traits using gene expression data from high FCR and low FCR birds. The complete expression matrix for the 21 samples was used in the study. After using dynamic tree cut algorithm, a total of 52 distinct co-expression modules including 32 to 1,267 genes per module were investigated. Based on hierarchical clustering some modules were grouped together and a total 38 modules (representing 10,562 genes) were kept and 1,267 uncorrelated genes were assigned into the grey module.

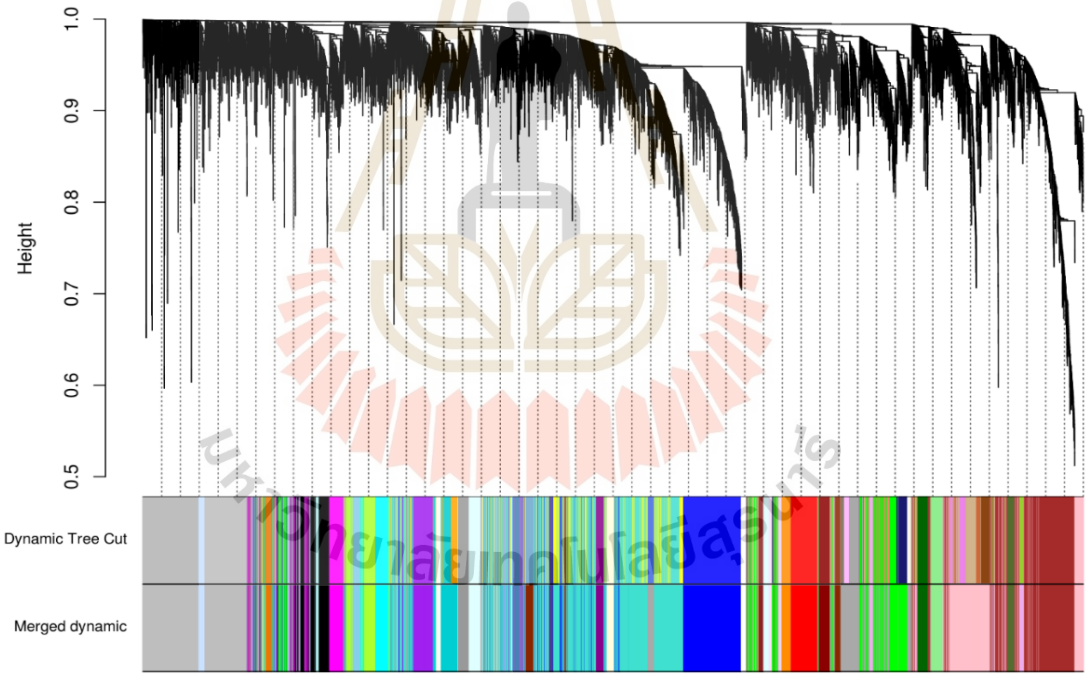


**Figure 5.4** Clustering dendrogram of gene profiles from thigh muscle tissues of Korat chicken at 10 weeks. The dendrogram was obtained by hierarchical clustering of adjacency based dissimilarity. The color row underneath the dendrogram shows the assigned original module and the merge modules.

(genes with no correlation to others) which was ignored in the following study (Figure 5.4). The number of genes for each of module is shown in Table 5.2.

**Table 5.2** The number of genes in each WGCNA module.

| Modules | Number of genes | Modules | Number of genes | Modules | Number of genes |
|---|---|---|---|---|---|
| MEdarkturquoise | 277 | MEthistle2 | 53 | MEpink | 860 |
| MElightcyan | 164 | MEdarkorange | 122 | MEivory | 73 |
| MEwhite | 121 | MEpaleturquoise | 114 | MEblue | 907 |
| MEdarkmagenta | 112 | MEblack | 329 | MEpurple | 266 |
| MEgrey60 | 152 | MElightyellow | 151 | MElightsteelblue1 | 79 |
| MEsalmon4 | 32 | MEdarkorange2 | 66 | MEmediumpurple3 | 82 |
| MEdarkslateblue | 58 | MEred | 340 | MEsteelblue | 116 |
| MEorangered4 | 190 | MEdarkred | 148 | MEdarkolivergreen | 112 |
| MEdarkgrey | 718 | MEbrown4 | 62 | MEskyblue | 118 |
| MEgreen | 681 | MEplum2 | 166 | MElightgreen | 152 |
| MEmagenta | 289 | MEdarkgreen | 145 | MEcyan | 207 |
| MEgreenyellow | 238 | MEgrey | 1267 | MEturquoise | 1886 |
| MElightcyan1 | 73 | MEthistle1 | 48 | MEbrown | 855 |

### 5.4.4 Feed efficiency and meat quality traits released module in the thigh muscle

The correlations between the 38 modules and phenotypic traits (feed efficiency and meat quality) are reported in Figure 5.5. This study found three modules (MEmediumpurple3, MEsteelblue and MEskyblue) that are significantly associated to feed efficiency (FCR at 10 weeks, RFI at 10 weeks) and some meat

parameters ($P<0.05$). Among the three modules, the MEmediumpurple3 is associated to FCR 10 weeks, RFI 10 weeks, WHC, IMP, AMP and Inosine in thigh muscle ($0.45<r<0.59$, $P<0.04$). This module contained the assembly competence domain (ACD) gene, baculoviral IAP repeat containing 5 (BIRC5) gene, cytochrome c oxidase assembly factor 3 (COA3) gene, myosin light chain 9 (MYL9) gene that may be involved in feed efficiency and meat quality in thigh muscle.

ACD is a conserved 29 residue sequence at the C-terminus of the sarcomeric myosin rod domain (coiled coil rod) that is required to allow the tails of myosin dimers to self-assemble into antiparallel arrays in the second assembly step of myosin heavy chain (MyHC) type II into filaments (Kachur and Pilgrim, 2008; Sohn et al., 1997). The study of Ikebe et al. (2001) reported that different ACD regions were important for the filament formation of smooth muscle and non-muscle myosin II. Also, the study of Dahl-Halvarsson et al. (2017) suggested that the mutation of L1793P which it was situated not far from the ACD gene might cause other primary effects on myosin and muscle function in human.

BIRC5 gene is the target gene of miR-133a (muscle-related miRNA in mammals) and miR-133a associated with skeletal muscle development in breast and thigh of chicken (Wang et al., 2013). According to Zhu et al. (2018) BIRC5 gene was found to be enriched during cell division, chromosome segregation and inflammatory response in mouse. It was suggested that BIRC5 might be a critical gene in the effects of microbiota on intestinal health. In agreement, Neufert et al. (2010) showed that the BIRC5 can limit bacterial growth and thereby contribute to mucosal wound healing.

COA3 is a mitochondrial membrane protein which it required for negative feedback regulation of COX1 translation in mitochondria. COA1 and COA3

link Shy1 to make an early assembly intermediates of COX1 (Mick et al., 2011; Mick et al., 2010). Also, they reported that the lack of COA3 function traps Mss51 in the committed state and promotes Cox1 synthesis. In human, hCOA3 stabilizes COX1 and supported its assembly with COX partner subunits which is associated with mitochondrial diseases (Clemente et al., 2013).

MYL9 is a regulatory light chain involving stability of myosin II and cellular integrity. It could interact with a variety of non-muscle types of MHC II (Park et al., 2011). MYL9 is associated with development of muscle fibers in chicken (Ye et al., 2017). In mice, knockout MYL9 gene resulted in a disorder of gastrointestinal motility (Gao et al., 2013).

Considering the function for all of genes, we could hypothesize that mutations of those genes might influence feed efficiency and meat quality between high FCR and low FCR chicken.

### 5.4.5 Functional enrichment analysis for modules of interest

MEmediumpurple3 module was selected to analyze the functional gene ontology enrichment analysis. For GO term enrichment analysis, we found 24 GO terms that were significantly enriched ($P<0.01$) (Table 5.3). As shown in Figure 5.6, mitochondrial gene expression, mitochondria respiratory chain complex assembly, mitochondrial translation and positive regulation of mitochondrial translation that may play a crucial role in feed efficiency and meat quality in slow-growing thigh muscle. The nuclear origin of the mitochondrial translation and respiratory chain function defect could be an effect on gene regulation of muscle led to decrease energy availability for muscle cell processes (Sasarman, et al., 2002). This is in agreement with the study of Rochard et al. (2000) that reported that an inhibition of

mitochondrial translation in avian myoblast cell line (QM7) is associated with a potent block of myoblast differentiation involving the development and regeneration of skeletal muscles to form multinucleated, contractile muscle fibers. Moreover, the study of Bottje et al. (2006); Bottje and Carstens (2009) reported a link between mitochondrial bioenergetics and dynamics and feed efficiency in broiler chickens. Chickens with low feed efficiency showed lower mitochondrial electron transport chain coupling and higher hydrogen peroxide compared to high feed efficiency. In counterpart, this led to a decrease in protein expression between low and high feed efficiency in mitochondria. The high feed efficiency birds were also reported to waste energy as heat and thereby have an inefficient link with inner mitochondrial membrane (RAIMBAULT et al., 2001). Also, they had greater fatty acid oxidation and cholesterol biosynthesis that led to high accumulation reactive oxygen species (ROS) in meat and oxidative stress, thereby the muscle altered nucleotide metabolism and mitochondrial dysfunction (Abasht et al., 2019; Petracci et al., 2019). Therefore, the sensitivity of oxidative stress in chicken might lead to lose the water retention ability and mitochondrial activity in muscle.

**Figure 5.5** Relationships between modules and feed efficiency and meat quality traits in the thigh muscle of KR chicken. Each row in the table corresponds to a module and each column to a trait. Each cell contains the corresponding correlation in the first line and the P-value in the second line. The table is color-coded by correlation, in accordance with the color legend. Intensity and direction of correlations are indicated on the right side of the heatmap (red, positively correlated; green, negatively correlated). The module name is shown on the left side of each cell.

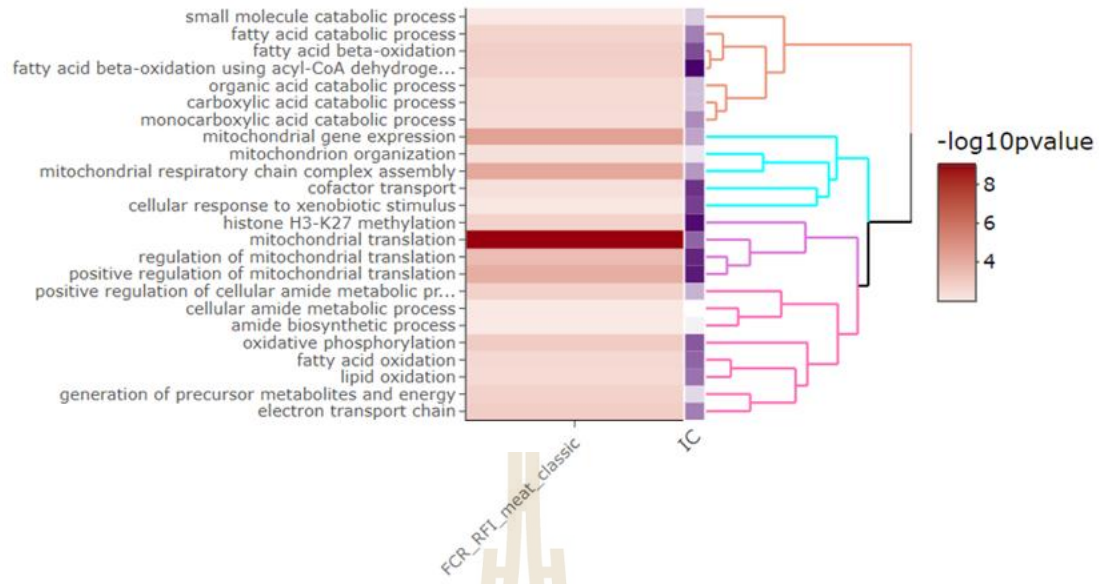


**Figure 5.6** Heat map of enriched 24 GO terms from Wang distance clustering of MEmediumpurple3 module.

**Table 5.3** Enriched GO terms from MEmediumpurple3 module involved feed efficiency and meat quality in thigh muscle.

| GO Name | FCR_RFI_meat gene frequency | P-value | Genes symbol |
|---|---|---|---|
| Generation of precursor metabolites and energy | 4.76% (5/105) | 0.002 | ATP5O, C26H6ORF125, NDUFV2, ETFDH, ETFRF1 |
| Oxidative phosphorylation | 12% (3/25) | 0.001 | ATP5O, C26H6ORF125, NDUFV2 |
| Fatty acid beta-oxidation | 11.11% (3/27) | 0.001 | HSD17B4, ACADS, ETFDH |
| Mitochondrion organization | 3.14% (6/191) | 0.005 | CHCHD6, C26H6ORF125, NDUFB9, AARS2, NDUFB5, COA3 |
| Fatty acid catabolic process | 9.68% (3/31) | 0.002 | HSD17B4, ACADS, ETFDH |
| Organic acid catabolic process | 5.48% (4/73) | 0.003 | HSD17B4, MTHFS, ACADS, ETFDH |
| Fatty acid oxidation | 8.82% (3/34) | 0.003 | HSD17B4, ACADS, ETFDH |
| Electron transport chain | 11.54% (3/26) | 0.001 | NDUFV2, ETFDH, ETFRF1 |
| Mitochondrial translation | 16.13% (5/31) | 0.000 | C26H6ORF125, AARS2, QRSL1, RPUSD4, COA3 |
| Mitochondrial respiratory chain complex assembly | 9.43% (5/53) | 7.00E-05 | C26H6ORF125, NDUFB9, AARS2, NDUFB5, COA3 |
| Fatty acid beta-oxidation using acyl-CoA dehydrogenase | 28.57% (2/7) | 0.001 | ACADS, ETFDH |
| Positive regulation of cellular amide metabolic process | 6.45% (4/62) | 0.002 | C26H6ORF125, CYR61, RPUSD4, COA3 |
| Lipid oxidation | 8.33% (3/36) | 0.003 | HSD17B4, ACADS, ETFDH |

**Table 5.3** Continue.

| GO Name | FCR_RFI_meat gene frequency | P-value | Genes symbol |
|---|---|---|---|
| Cellular amide metabolic process | 2.22% (8/360) | 0.009 | MTHFS, C26H6ORF125, AARS2, QRSL1, RPS3A, CYR61, RPUSD4, COA3 |
| Amide biosynthetic process | 2.41% (7/291) | 0.009 | C26H6ORF125, AARS2, QRSL1, RPS3A, CYR61, RPUSD4, COA3 |
| Small molecule catabolic process | 3.92% (4/102) | 0.010 | HSD17B4, MTHFS, ACADS, ETFDH |
| Carboxylic acid catabolic process | 5.48% (4/73) | 0.003 | HSD17B4, MTHFS, ACADS, ETFDH |
| Cofactor transport | 15.38% (2/13) | 0.005 | SLC48A1, TCN2 |
| Regulation of mitochondrial translation | 17.65% (3/17) | 0.000 | C26H6ORF125, RPUSD4, COA3 |
| Positive regulation of mitochondrial translation | 25% (3/12) | 0.000 | C26H6ORF125, RPUSD4, COA3 |
| Histone H3-K27 methylation | 25% (2/8) | 0.002 | CHD3, SUPT6H |
| Cellular response to xenobiotic stimulus | 11.76% (2/17) | 0.008 | NR3C1, CRYZ |
| Monocarboxylic acid catabolic process | 7.89% (3/38) | 0.004 | HSD17B4, ACADS, ETFDH |
| Mitochondrial gene expression | 10.64% (5/47) | 4.00E-05 | C26H6ORF125, AARS2, QRSL1, RPUSD4, COA3 |

## 5.5 Conclusions

This study revealed new genetic information on the pathways and genes involved in feed efficiency and meat quality in thigh slow-growing chicken. Thigh of low FCR chicken had higher lipid oxidation and protein oxidation than thigh of high FCR chicken. No gene was differentially expressed between high FCR and low FCR birds in thigh muscle. However, according to WGNCA analysis, mitochondrial gene expression, mitochondria respiratory chain complex assembly, mitochondrial translation and positive regulation of mitochondrial translation pathways as well as the 4 genes: ACD, BIRC5, COA3, MYL9 might play a crucial role in feed efficiency and meat quality in slow-growing thigh muscle.

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# CHAPTER VI

# CONCLUSIONS AND RECOMMENDATION

## 6.1 Conclusion

This study provided new information on the phenotypic correlations between feed efficiency (FCR, RFI) and meat quality in breast and thigh muscle in male slow-growing chicken. According to breast muscle, FCR was likely to impair breast (water retention ability) while, the correlation between RFI and technological meat was weak. Regarding to thigh muscle, FCR and RFI could not impact on biochemical compound changing. FCR could impact on water retention ability, flavor precursor and also RFI could be affected on flavor precursor. Moreover, this study revealed the genes and pathways involved in feed efficiency and meat quality in thigh muscle. Mitochondrial gene expression, mitochondria respiratory chain complex assembly, mitochondrial translation and positive regulation of mitochondrial translation pathways as well as the 4 genes: ACD, BIRC5, COA3, MYL9 might play a crucial role in feed efficiency and meat quality in thigh muscle of the KR chicken thigh.

## 6.2 Recommendation

The results suggested that RFI is suitable criteria to improve feed efficiency without interfering meat quality in male slow-growing KR chicken. The next step would be confirmed the information by genetic correlation on larger data set. In addition, the correlation between RFI and meat quality in breast and thigh of female

slow-growing KR chicken should be investigated.

Regarding enrichment functional analysis suggested that most genes involved in feed efficiency, flavor precursor (IMP, AMP, inosine) and also water retention ability are functioned in the mitochondrial activity and lipid oxidation pathways. Therefore, roles of these genes in the pathways should be investigated for deeper understanding. Moreover, consequence of genetic improvement for superior RFI, particularly in term of sensitivity of oxidative stress of the chicken should be concerned and investigated for further study.

# BIOGRAPHY

Miss Chotima Poompramun was born on 27th May 1987 in Mahasarakham, Thailand. In 2009, she graduated a Bachelor degree in Animal Production of Technology Suranaree University of Technology, Thailand. In 2013, She graduated a Master's degree in Animal Production of Technology Suranaree University of Technology, Thailand. She studied in the field of Animal Technolgy and Innovation at School of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology from October 2013 to July 2019 with the thesis entitled "Identification of genes involved in feed efficiency and meat quality of Korat chicken". The partly results of her Ph.D. thesis have been present in the Doctoral and post-doctoral students day conference, 2018 at Nouzilly, France, The 6th Meat production success based on innovation technology on June18-19, 2018 at Bangkok, Thailand, The 9th Annual SLRI User Meeting 2019: AUM2019 on May 1, 2019 and The the 2nd International Conference on Native Chicken 2019 (ICONC 2019) on July 9-12, 2019 at NakhonRatchasima, Thailand.