PROTEOMIC PROFILES INVOLVED IN THE TRAIT OF FEED EFFICIENCY OF KORAT CHICKEN

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ไก่โคราช จัดเป็นไก่กลุ่มโตช้าที่มีคุณภาพเนื้อที่โดดเด่น เช่น เนื้อเหนียวนุ่ม รสชาติดี และมีสารที่มีประโยชน์ต่อร่างกาย แต่อย่างไรก็ตามการที่ไก่โคราชมีประสิทธิภาพการใช้อาหารที่ต่ำส่งผลทำให้ต้นทุนการผลิตสูงขึ้น จึงมีความจำเป็นอย่างยิ่งที่จะต้องปรับปรุงพันธุกรรมของไก่โคราชให้มีประสิทธิภาพการใช้อาหารที่สูงขึ้นเพื่อลดต้นทุนการผลิต และเพิ่มความสามารถในการแข่งขันให้กับผู้ผลิตไก่ในประเทศไทย ดังนั้นการวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาความแตกต่างของโปรติโอมิกส์โปรไฟล์, การแสดงออกของโปรตีน และกลไกที่เกี่ยวข้องกับลักษณะประสิทธิภาพการใช้อาหารในเนื้ออก เนื้อสะโพก และลำไส้ส่วนต้น (duodenum) โดยในการศึกษาแรกไก่โคราชเพศผู้ทั้งหมด 75 ตัว ถูกนำมาเลี้ยงในกรงเดี่ยว เพื่อคำนวณความสามารถในการใช้อาหาร (FCR และ RFI) จากข้อมูลปริมาณที่กินได้ และน้ำหนักตัว เมื่อไก่โคราชมีอายุได้ 10 สัปดาห์จึงทำการเก็บตัวอย่างลำไส้ duodenum เพื่อนำไปวิเคราะห์ทางโปรติมิกส์ด้วยเทคนิค label-free quantitative proteomic ซึ่งข้อมูลของไก่ที่มีค่า FCR สูงที่สุด (n=3) และต่ำที่สุด (n=3) ถูกนำไปใช้สำหรับการวิเคราะห์ทางสถิติ อย่างเช่น การหาความแตกต่างของการแสดงออกของโปรตีน (differential abundant proteins), เครือข่ายปฏิสัมพันธ์ระหว่างโปรตีน (protein-protein interaction networks), การค้นหาหน้าที่ของกลุ่มโปรตีน (functional enrichment) และการวิเคราะห์บาทวิถี (pathway analysis) จากผลการศึกษาพบว่า มีโปรตีนทั้งหมด 40 ชนิดที่มีการแสดงออกที่แตกต่างกันระหว่างไก่สองกลุ่ม โดยกลุ่มโปรตีนเหล่านี้มีบทบาทหน้าที่ในกลไกที่เกี่ยวข้องกับกระบวนการ glycolysis/gluconeogenesis, peroxisome, oxidative phosphorylation, tight junction และ cysteine and methionine metabolism ดังนั้นการค้นพบโปรตีนเหล่านี้มีความเป็นไปได้ในการนำไปใช้สำหรับเป็น biomarker เพื่อคัดเลือกให้ไก่โคราชมีประสิทธิภาพการใช้อาหารให้ดีขึ้น

ถึงแม้ว่าการค้นพบในการศึกษาแรกนั้นจะชี้ให้เห็นถึงความเป็นไปได้การปรับปรุงพันธุ์ให้ไก่กลุ่มโตช้านั้นมีประสิทธิภาพการใช้อาหารที่ดียิ่งขึ้น แต่อย่างไรความเข้าใจถึงผลกระทบจากการคัดเลือกต่อคุณภาพเนื้อของไก่กลุ่มโตช้ายังไม่ชัดเจน ดังนั้นวัตถุประสงค์ในการศึกษาต่อมาจึงมีเพื่อที่จะศึกษาถึงความสัมพันธ์ของลักษณะประสิทธิภาพการใช้อาหารและคุณภาพเนื้อของไก่โคราช โดยข้อมูลของประสิทธิภาพการใช้อาหาร (FCR และ RFI) คุณภาพเนื้อ และโปรติโอมิกส์โปรไฟล์ในเนื้อสะโพกถูกนำมาใช้

สำหรับการวิเคราะห์ weighted gene co-expression network analysis (WGCNA) จากผลการศึกษา พบว่า โมดูลกลุ่มของโปรตีน 127 ชนิดที่อยู่ใน turquoise module มีความสัมพันธ์กับลักษณะประสิทธิภาพการใช้อาหารและคุณภาพของเนื้อสะโพก ซึ่งพบว่ากลุ่มโปรตีนเหล่านี้มีบทบาทหน้าที่เกี่ยวข้องกับกลไก glycolysis/gluconeogenesis, metabolic pathway, carbon metabolism, biosynthesis of amino acids, pyruvate metabolism และ protein processing in the endoplasmic reticulum แต่อย่างไรก็ตามความสัมพันธ์ของทั้งสองลักษณะกลับมีทิศทางที่ตรงกันข้ามกัน เมื่อประสิทธิภาพการใช้อาหารเพิ่มขึ้นส่งผลให้คุณภาพของเนื้อสะโพกลดลงด้วยการเปลี่ยนแปลงการทำงานของกลไกดังกล่าว เห็นได้จากค่าของ water holding capacity ในเนื้อลดลง และมีปริมาณ inosine content, amide I และ CH-bending เพิ่มขึ้น นอกจากนี้ยังพบว่าโปรตีนทั้ง 10 ตัวนี้ TNNT1, TNNT3, TNNI2, TNNC2, MYLPF, MYH10, GADPH, PGK1, LDHA และ GPI มีบทบาทสำคัญต่อทั้งสองลักษณะที่สนใจ ดังนั้นผลการศึกษาชิ้นนี้ชี้ให้เห็นว่าในการออกแบบโปรแกรมสำหรับการคัดเลือกสัตว์นั้นมีความจำเป็นอย่างมากที่ต้องพิจารณาทั้งสองลักษณะควบคู่กันเพื่อให้แน่ใจได้ว่าเมื่อไก่มีประสิทธิภาพการใช้อาหารที่ดีขึ้น คุณภาพเนื้อจะยังไม่เปลี่ยนแปลงไป

สาขาวิชาเทคโนโลยีและนวัตกรรมทางสัตว์ ลายมือชื่อนักศึกษา _____
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PRAMIN KAEWSATUAN : PROTEOMIC PROFILES INVOLVED IN THE TRAIT OF FEED EFFICIENCY OF KORAT CHICKEN. THESIS ADVISOR: ASSOC. PROF. DR. AMONRAT MOLEE, Ph. D., 101 PP.

Keyword: SLOW-GROWING CHICKEN/FEED EFFICIENCY/MEAT CHARATERISTICS/LABEL-FREE PROTEOMICS/BIOINFORMATICS

The Korat chicken (KR), developed in Thailand, is a slow-growing breed developed as an alternative breed for Thai chicken producers. The growing interest in slow-growing chicken meat, due to its unique taste, distinct texture, health benefits, and higher broiler welfare has led to higher market demand for KR. Understanding the molecular mechanism regulating feed efficiency (FE) allows for designing a suitable selection program and contributing to breeding more efficient chicken for poultry production. Thus, the objective of our study was to investigate the proteome differences and possible pathways associated with FE in male KR using a label-free quantitative proteomic approach. Seventy-five KR males were individually evaluated for FE, and duodenum samples from 6 animals (3 high-FE and 3 low-FE chickens) were collected at 10 weeks of age for differential abundant proteins (DAPs), protein-protein interaction networks, functional enrichment, and pathway analysis. We found 40 DAPs significantly associated with FE pathways, including glycolysis/ gluconeogenesis, peroxisome, oxidative phosphorylation, tight junction, and cysteine and methionine metabolism. Thus, the variations in observed DAPs or genes related to DAPs could be interesting biomarker candidates for selection for higher feed utilization efficiency in chickens.

Although improving FE can be achieved through genetic selection, the impact of selection for FE on meat characteristics is largely unknown. Thus, the investigation insight into the molecular mechanism underlying FE and meat characteristics traits is required. In our second study, therefore, we used weighted gene co-expression network analysis (WGCNA) to identify the key protein modules and pathways in the thigh muscle. The results revealed that the same proteins contained in the turquoise module significantly correlated both FE and thigh meat characteristics and mostly enriched in the pathways including glycolysis/gluconeogenesis, metabolic pathway,

carbon metabolism, biosynthesis of amino acids, pyruvate metabolism, and protein processing in the endoplasmic reticulum. However, the correlation was unfavorable; improving FE may result in a decrease in meat quality as a result from reduced water holding capacity, increased inosine content, amide I, and CH-bending through the alteration of these identified pathways. In addition, the 10 hub proteins of the significant module including TNNT1, TNNT3, TNNI2, TNNC2, MYLPF, MYH10, GADPH, PGK1, LDHA, and GPI were identified. Given that the same proteins and pathways are present in FE and meat characteristics, but in opposite directions, selection practices for KR should take into account both trait groups simultaneously to maintain the high quality of KR meat while improving FE.

School of Animal Technology and Innovation Student's Signature
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LIST OF ABBREVIATIONS

M	=	Molar
mM	=	Milli Molar
mg	=	Milligram
µg	=	Microgram
g	=	Relative centrifugal fields
min	=	Minute
mL	=	Milliliter
℃	=	Degree Celsius
Wk	=	Week
ppm	=	Parts per million
FC	=	Fold change
h	=	Hour
DEGs	=	Differential expression genes
DAPs	=	Differential abundance proteins
SNPs	=	Single nucleotide polymorphism
FE	=	Feed efficiency
FI	=	Feed intake
ADG	=	Average daily gain
BW	=	Body weight
BWG	=	Body weight gain
KR	=	Korat chickens
FCR	=	Feed conversion ratio
RFI	=	Residual feed intake
DL	=	Drip loss
SF	=	Shear force
TL	=	Thawing loss
WHC	=	Water holding capacity
pHu	=	Ultimate pH

LIST OF ABBREVIATIONS (Continued)

IMF	=	Intramuscular fat
PSE	=	Pale Soft Exudative
DFD	=	Dark Firm Dry
IMP	=	Inosine-5’monophosphate
GMP	=	Guanosine 5'-monophosphate
ATP	=	Adenosine triphosphate
ADP	=	Adenosine diphosphate
AMP	=	Adenosine monophosphate
Ino	=	Inosine
Hx	=	Hypoxanthine
FT-IR	=	Fourier transform infrared spectroscopy
LC-MS	=	Liquid chromatography-mass spectrometry
KEGG	=	Kyoto Encyclopedia of Genes and Genomes
GO	=	Gene ontology
AMBIC	=	Ammonium bicarbonate buffer
DTT	=	Dithiothreitol
IAA	=	Iodoacetamide
HCD	=	Higher-energy collisional dissociation
DDA	=	Data-dependent acquisition
FDR	=	Adjusted P-values
ROS	=	Reactive oxygen species
WGCNA	=	Wight Gene Co-expression Network Analysis
PS	=	Protein significance
MM	=	Module membership

CHAPTER I
INTRODUCTION

1.1 Introduction

Consumers are increasingly interested in meat quality, safety, and animal welfare. These trends favor slow growing chickens due to the breeds' meat characteristics, including unique taste, firmer texture, higher nutritional value, and welfare compared to fast-growing commercial breeds (Lusk, 2018). In Thailand, Korat chicken (KR), was developed as an alternative meat-type chicken for producers. Korat chicken is a crossbreed between the Thai indigenous Leung Hang Khao chicken and the Suranaree University of Technology (SUT) synthetic line. Despite its good meat characteristics, the growth rates and feed efficiency (FE) of KR are low, causing low profits (Hang et al., 2018). Thus, to increase its competitiveness in the Thai poultry production market and to offer an efficient alternative breed for small- to moderate-sized Thai farms, improving FE is the most important breeding goal in KR chicken.

Traditionally, FE is measured as feed conversion ratio (FCR); a ratio of feed intake (FI) to body weight gain (BWG). The well-known limitation of FCR is that selection for FCR improves more efficiently numerator trait (FI) compared to denominator trait (BWG) causing nonlinear selection pressure (Gunsett, 1984). Another commonly used FE measurement is residual feed intake (RFI); a difference between observed and expected feed intake based on requirements for maintenance and growth (Koch et al., 1963). Due to its low genetic correlation with production traits and a moderate correlation with FCR and FI, it has been suggested that RFI is the most appropriate trait for the genetic improvement of energy efficiency in poultry (Willems et al., 2013; Xu et al., 2016). Since the direct selections of FCR and RFI impacted on different traits, therefore, the study on molecular mechanism related to both FCR and RFI regulation is needed.

In addition, FE depends on feed intake, energy homeostasis, intestinal structure, and many physiological processes related to the utilization of feed, including

intestinal nutrient digestion, absorption, the integrity of the intestinal epithelium, and translocation of intestinal antigens (Richards and Proszkowiec-Weglarz, 2007; Choct, 2009; Nain et al., 2012). Previous studies have shown that high-FE chickens have longer gastrointestinal tracts (Kadhim et al., 2010; Krás et al., 2013; Mabelebele et al., 2014), higher nutrient digestibility (Rougière et al., 2009; De Verdal et al., 2010), and larger duodenal absorptive villi surface (Nain et al., 2012) than low-FE chicken. The duodenum is a complex organ with an important role in FE, as it regulates the feed digestion process and energy homeostasis (Recoules et al., 2019). Although there are many studies focus on discovering candidate genes of FE trait, but a few of them have been unidentified on gene functional level of slow-growing chicken and their key mechanism remain unknown. Since the relationship between gene expression levels and their corresponding protein abundance is indirect and the physiological processes are mainly controlled by protein levels (Burgess, 2004) the knowledge from genomic and transcriptomic studies may not be enough to explain the genetic basis of FE. Therefore, proteomic analysis may provide additional insight into the functional mechanisms underlying FE (Kong et al., 2016a; Fu et al., 2017; Fonseca et al., 2019). Currently, little is known the association between FE traits and proteomics in the duodenal tissues of chicken. Thus, chapter 3's findings can fulfill this gap, revealing the alteration of duodenal metabolic processes influences the FE variation.

However, it has been found that improving FE may cause decrease in meat quality and reduce consumer acceptance of the meat (Zhou et al., 2015). Previous skeletal muscle transcriptomic analysis in chickens have indicated that FE may be related to nucleotide sugar biosynthesis, glycogen metabolism, and lipid uptake and transport (Abasht et al., 2019). Our previous transcriptomic analysis (Poompramun et al., 2021) indicated that nucleotide metabolism, fatty acid metabolic process, and oxidative stress play a key role in the regulation of both FE and the quality of thigh meat, suggesting that improving FE can have negative impact on meat texture and the nutritional values of the meat via activating the accumulation of biochemical compounds and flavor indicators. Although some molecular mechanisms have been extensively investigated in transcriptome level (Poompramun et al., 2021), understanding how FE and meat quality in the thigh of the slow-growing chicken are linked in the proteomics level is missing. Overall, a better understanding of how FE

affects meat characteristics is crucial to avoid any unfavorable effects of improving FE on quality and characteristics of thigh meat of KR. The results in the chapter 4 revealed key hub proteins and pathways that regulate both FE traits and meat characteristics, flavor, and biochemical compound in KR thigh meat, indicating the effect of FE selection on meat characteristics. This finding could be used to assess the direction and limitations of chicken breeding for improving feed efficiency and the texture, flavor, and nutritional value of meat chicken

1.2 Research objectives

The objectives of this study were:

1.2.1 To identify proteomic profiles that differ between high- and low- FCR of Korat chicken in breast, thigh, and small intestine.

1.2.2 To identify differential expression proteins that differ between high- and low-FCR of Korat chicken in breast, thigh, and small intestine.

1.2.3 To identify active pathways that differ between high- and low- FCR of Korat chicken in breast, thigh, and small intestine.

1.2.4 To identify key proteins and pathways involved in feed efficiency and meat characteristics.

1.3 Research hypotheses

1.3.1 Korat chicken with different FCR will have different protein expression and pathways in breast, thigh, small intestine tissues.

1.3.2 Different feed efficiency may have an impact on meat quality variation.

1.4 Scope of the study

A total of 75 male KR is a target population of this study. Individual male FE measures included feed conversion ratio (FCR) and residual feed intake (RFI) from hatch to weeks 10 was calculated. The comparison of proteomic profile and differential abundance proteins in breast, thigh, and small intestine between high-FCR and low-FCR group of KR was first conducted, and key proteins were obtained. Then, bioinformatic analysis, i.e., gene ontology (GO), KEGG pathway, and protein-protein

interaction network, were used to clarify the functional role of the key proteins regulating feed efficiency.

After 24 h of chilling, the thigh meat samples were collected for meat quality measurement. In the present study, we measured those meat characteristics traits that are related to the texture and flavor of the thigh meat. The physiochemical traits included ultimate pH (pHu), water holding capacity (WHC), and drip loss (DL) The adenosine triphosphate-related traits included guanosine monophosphate (GMP), inosine monophosphate (IMP), adenosine monophosphate (AMP), and inosine (Ino), Moreover, the biomolecule profiles of lipids, amide I, amide II, amide III, CH-binding of lipid, carbohydrate and glycogen obtained from the Fourier Transform Infrared (FTIR) were used to reveal any changes in key biomolecules related to FE.

Wight Gene Co-expression Network Analysis (WGCNA), a powerful bioinformatics approach, were also employed to investigate the correlations between feed efficiency and meat characteristics in the thigh meat of male KR chicken. This could lead to variation in meat characteristics and age dependent factors upon protein abundance profile and their related pathways between high-FCR and low-FCR of KR.

1.5 Expected benefits

The knowledge of this study advances our molecular understanding of key biological processes regulating feed efficiency and its consequence on other economical traits which can pave the way for the development of suitable genetic selection

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CHAPTER II

LITERATURE REVIEWS

2.1 The world population prospects and world's situation of feedstuffs.

The global population is currently growing rapidly (Figure 2.1). This indicates rapid changes in social and economic aspects, as well as a greater appreciation for high-quality proteins, resulting in an increase in global food demand, especially of protein. Among the main types of meat produced in the world, poultry has recorded the greatest absolute and relative growth rate over the last 50 years (Figure 2.2). This trend has been driven mainly by the greater affordability of chicken meat compared to other meats, the convenience and potential health benefits, and religious and cultural concerns (Baldi, Soglia, and Petracci, 2021). Thus, the poultry sector will play a crucial role in ensuring food security for an increasing world population (Mottet and Tempio, 2017).

In addition, cost of feed is not stable representing up to 70% of the total cost of poultry farming system. Thus, feed costs are classified a main factor affecting the economic sustainability. From report of the world bank, (2012) indicated heat and lack of rainfall were important factors that effect on the abrupt price increase of feedstuffs (Figure 2.3). Moreover, world's agricultural land has decreased continuously (Figure 2.4) that also effect on feedstuffs price. Therefore, improving the ability of chicken body to convert their feed into body mass or edible products, which is generally known as the efficiency of feed utilization or feed efficiency (FE), represents a more sustainable and efficient way to minimize feed cost and increase profitability of the poultry production.

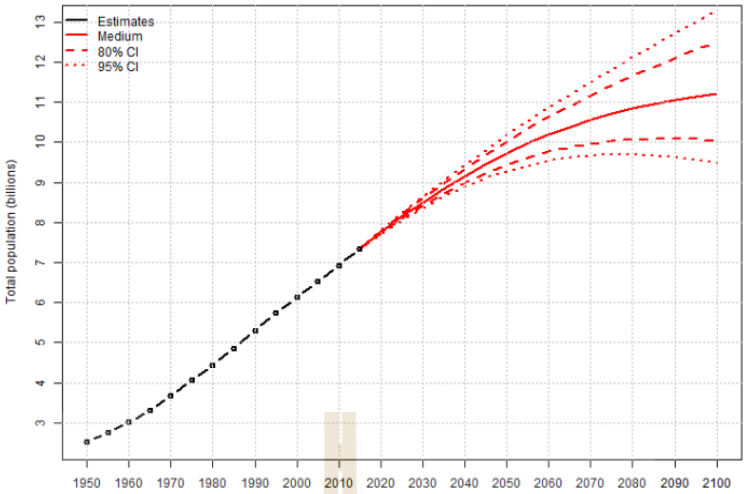

Figure 2.1 World population prospects 1950-2100 years.

Source: United nations department of economic and social affairs population division (2015).

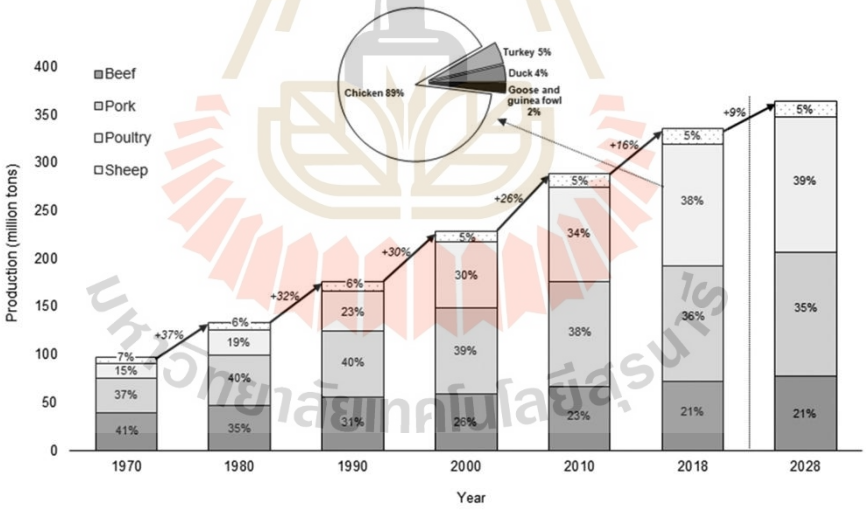

Figure 2.2 The production of the four main types of meat produced in the world (beef, pork, poultry and sheep) trend from 1970-2028.

Source: Zampiga et al. (2021).

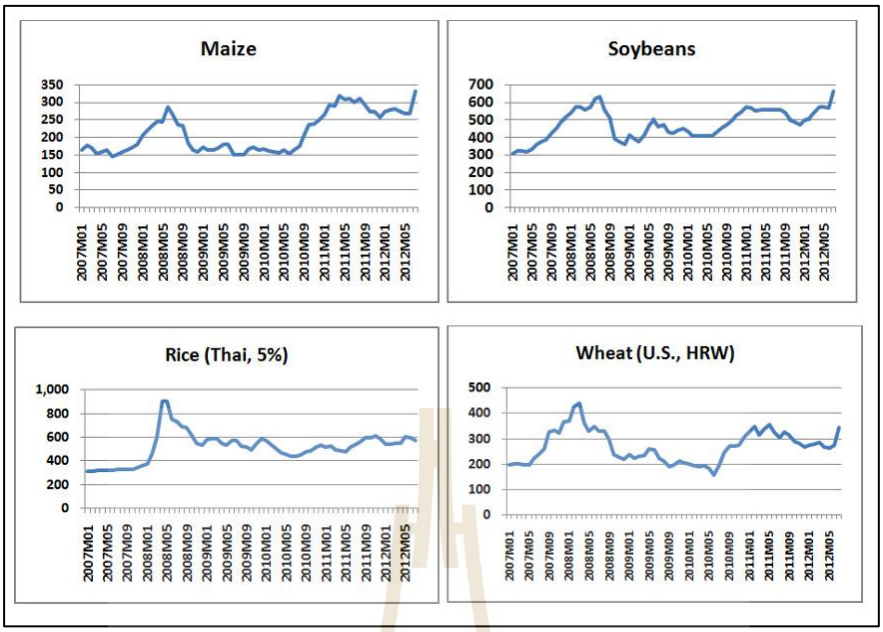

Figure 2.3 Price of feedstuffs trend from 2007-2015 years.

Source: http://siteresources.worldbank.org/EXTPOVERTY/Resources/336991-1311966520397/Food-Price-Watch-August-2012.html.

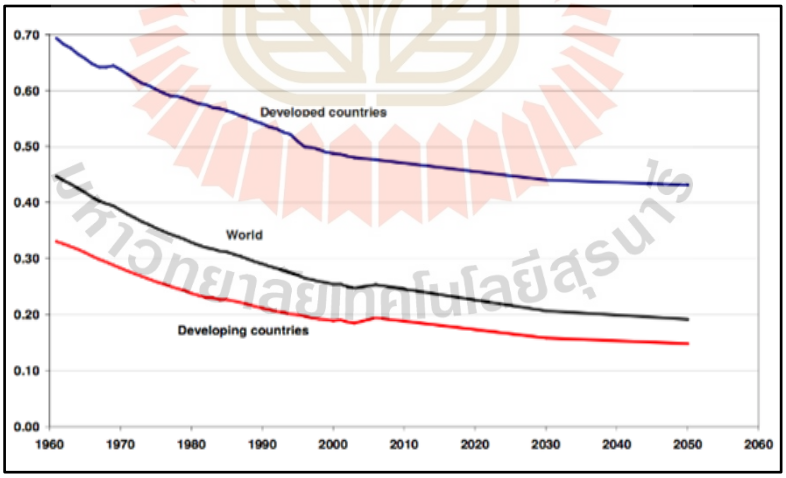

Figure 2.4 Agricultural land use change in the last 50 and the next 50 years.

Source: https://ourworldindata.org/land-use-in-agriculture/#arable-land-per-capita-ha-in-use-per-person-1961-2050-jelle-bruinsma-2009-faoref.

2.2 The introduction of slow growing chicken and Korat chicken

Most of the chicken meat currently available on the global market is fast-growing chicken, also known as broiler (a term for chickens raised for meat). This breed has been bred to grow faster and bigger than they were a decade ago (Figure 2.5). This makes broilers more affordable to consumers and more sustainable for chicken production because they require less feed and use fewer natural resources to grow. Despite being favored by producers due to its high feed efficiency, rapid growth is the cause of several unintended results (Tavárez et al., 2016). Some research reported that fast growing chickens have serious leg problems and pain, so they are unable to stand and support their own weight, preventing their natural behavior (Corr et al., 2003; Dixon, 2020). In addition, these fast-growing chickens not only have poor welfare but also produce lower-quality meat with a higher prevalence of wooden breast, white striping, and muscle disorders (Lusk et al., 2018). Regarding these phenomena, there is increased pressure on all sectors of chicken production because of shifting consumer perceptions and demands.

A growing number of consumers who are concerned about animal welfare, nutrition, and sustainability may be the reason that fast-growing chicken are no longer able to meet consumer demands (Wilhelmsson et al., 2019). As a result, chicken companies have been urged to switch to slower-growing chicken for better taste and welfare (Strom, 2017). It has been previously reported that slow-growing breed produces meat that is more flavorful, tender, and less juicy than conventional chicken breeds (Napolitano et al., 2013). Moreover, slow growing chicken has been raised under better welfare condition, allowing them to mature properly. Consequently, a high consumption of slow-growing chicken meat product was observed particularly in East Asia (Quentin et al., 2003; Rizzi et al., 2007), as well as a growing consumer awareness of slow-growing breed meat products in some regions of the world. (Scott et al., 2017; Fisher, 2017; Davies, 2019).

Korat chicken (KR) is a crossbreed between the Thai indigenous Leung Hang Khao chicken and the Suranaree University of Technology (SUT) synthetic line and has a slow growth rate. This breed reach market weight in 10 weeks (1.2 kg) and a feed conversion ratio (FCR) of around 2.80 (Hang et al., 2018). KR meat contains less fat and more collagen than commercial broiler meat. It also has a firm and unique

texture, which attracted to Thai consumers more than broiler meat. Although KR has good meat characteristics and welfare, its low feed efficiency due to slower rate of growth results in more feed needing to be provided which leads to high feed cost. Regarding to Table 2.1 showing that the growth performance, feed efficiency of KR close to other slow-growing chicken (Label Rouge, France). Thus, the biggest challenge in developing a breed is not only slower growth but also improving feed efficiency to meet consumer demand and sustainability for chicken production.

Table 2.1 The summary of feed efficiency and growth performance in chickens.

Breed	**FCR (g:g)**	**RFI (g)**	**BW (g)**	**ADG (g/day)**	**References**
Broiler (42 days)	1.89±0.09	0.00±5.69	2499.08± 166.43	88.89±8.55	Li et al. (2020)
Slow-growing hybrid (Rowan Ranger)	2.90±0.04	-	2656.00 ± 120.20	-	Rezaei et al. (2018)
Label Rouge (70 days)	3.15±0.40	0.62±91.71	1803.00 ±270.30	-	N'Dri et al. (2006)
Thai native chicken (84 days)	4.66	-	1156.05	16.56	Jaturasitha et al. (2002)

FCR = feed conversion ratio, RFI = residual feed intake, BW = body weight, ADG = average dairy gain

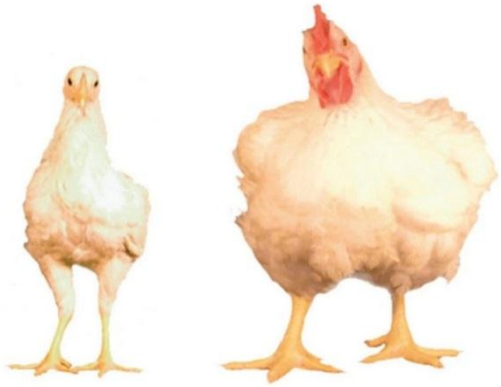

Figure 2.5 A commercial broiler genotype produced in the 1950s (left) and a commercial broiler genotype produced in 2005 (right).

Source: Tallentire et al. (2016).

2.3 Defining of feed efficiency

In poultry, FE is traditionally measured as feed conversion ratio (FCR); a ratio of feed intake (FI) to body weight gain (BWG). Another commonly used FE measurement is residual feed intake (RFI); a difference between observed and expected feed intake based on requirements for maintenance and growth (Koch et al., 1963). These two indexes were generally used to determine the ability of chicken to convert feed to product. Moreover, Zampiga et al. 2018 reported that the variations in FE are influenced by the energy homeostasis which is biological process involving the regulation of the balance point between energy intake (energy inflow), which determined by feed intake and the efficacy of digestive capacity. and expenditure (energy outflow), which depends on the rate of metabolic process or energy requirement for maintaining body weight and production. Consequently, a more efficient chickens showing a lower value of RFI and FCR indicating that less feed intake required for body weight gain, which possibly due to a greater digestive capacity accompanied with a more favorable nutrients partitioning towards anabolic processes (Zampiga et al. 2018). Some studies showed that gastrointestinal physiology in absorption system differed between FE line (Table 2.2). Thus, it indicated genetic effect is a main contributor to the differences in feed efficiency of chickens, in term of digestive capacity. Moreover, Previous studies have shown that

highFE chickens have longer gastrointestinal tracts (Kadhim et al., 2010; Krás et al., 2013; Mabelebele et al., 2014), higher nutrient digestibility (Rougiere et al., 2010; De Verdal et al., 2010), and larger duodenal absorptive villi surface (Nain et al., 2012) than low-FE chicken. The duodenum is a complex organ with an important role in FE, as it regulates the feed digestion process and energy homeostasis (Recoules et al., 2019).

Heritability for FCR has been reported in Arkansas broilers and the commercial slow-growing meat-type chicken line was 0.41 and 0.33, respectively (N'Dri er al., 2006; Aggrey et al., 2010). The estimated heritability of RFI for the laying hens was 0.47 (Wolc et al., 2013). Moreover, the study of Liu et al. (2017) showed the heritability estimates of FCR and RFI were 0.29 and 0.50, respectively. As abovementioned illustrates that both of FCR and RFI are moderately heritable in chicken, consequently, the efficient selection can be achieved using these two traits as the selection criterion. In the past year, genetic selection provided more practical and has been successful in improving FE by lowering FCR and RFI, because it eliminates the time-consuming task of collecting phenotypic data for all FE traits as is required to make genetic progress using traditional selection. The well-known limitation of FCR is that selection for FCR improves more efficiently numerator trait (FI) compared to denominator trait (BWG) causing nonlinear selection pressure (Gunsett, 1984). Due to its low genetic correlation with production traits and a moderate correlation with FCR and FI, it has been suggested that RFI is the most appropriate trait for the genetic improvement of energy efficiency in poultry (Willems et al., 2013; Xu et al., 2016). Given that FCR and RFI measures different components of FE, it is important to understand the biological basis of FCR and RFI given their complexity.

Table 2.2 Absorption system of high feed efficiency and low feed efficiency chicken.

Breed	Gastrointestinal tract						References
	Crop	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	
(70 days)	Weight (g)	Weight (g)	Weight (g)	Length (cm)	Length (cm)	Length (cm)	
Ross308	9.47±1.02[a]	7.48±0.85	38.42±3.21[a]	31.90±0.20	60.60±0.30	70.60±0.20	Mabelebele
Indigenous	54.80±8.50[b]	6.44±0.69	55.51±6.52[b]	25.00±1.00	47.50±0.20	55.60±0.10	et al. (2014)
Venda							
(23 days)							
Breed: D +	4.78±0.75	8.19±2.08**	26.0±4.20**	49.70±7.45	87.70±15.60**	84.20±16.10	Verdal et al. (2011a)
Breed: D –	4.74±0.77	6.72±1.39**	19.40±4.23**	51.10±7.45	93.30±14.90**	87.40±14.70	
Villus height (μm)							
Breed: D +				1367.00[a]	939.50[a]	678.80	Verdal et al. (2011b)
Breed: D –				1429.60[a]	1075.70[b]	727.00	
(23 days)							

a, b: Means within row carrying no common superscripts are significantly different at P<0.05, ** Means within row carrying no common superscripts are significantly different at P<0.01, ne: not estimated, D+ = efficient breed, D- = inefficient breed

2.4 The global gene expression study on feed efficiency

Over the last few decades, global gene expression studies have been conducted to gain a greater understanding of the cellular basis of the FE trait. The identification of several genes that are involved in different FE traits and genomic markers that could permit the detection of an animal's potential desired trait would be helpful for animal breeders to select the most appropriate production system, animal types, and markets.

It has been found that many studies focusing on genetic factors in terms of genetic structure and gene expression are related to the FE trait of chicken as showed in the table 2.3. They are using high-throughput technologies called genomic and transcriptomic technologies, such as RNA sequencing, microarrays, and genome-wide association studies (GWAS), to discover candidate gene makers. Previous studies found not only genes that related to muscle fiber growth and development but also genes that involved nutrient digestion and absorption in the small intestine, which is one of the indicators of the feed efficiency trait. The report of Ozaydin and Celik (2012) indicated the intestine villi of Hybro and Ross 308 strains were at different developmental stages on days 18, 20, and 21 of incubation. Chen et al. (2015) demonstrated that the mRNA levels of the Interleukin (IL)-8 gene, IL-1β gene, transforming growth factor (TGF)-β4 gene, fatty acid-binding protein 6 (FABP) gene, fatty acid-binding protein 2 (FABP2) gene, Occludin gene, Mucin 2 (MUC2) gene, PepT1 gene, EAAT3 gene, and SGLT1 gene were significantly different between the normal gut barrier group and gut barrier failure group of male Ross 308 broilers. Mott et al. (2008) discovered that the levels of gene expression (PepT1 gene, EAAT3 gene, and SGLT1 gene) in laying chickens with high and low feed efficiency were significantly different.

Overall, the knowledge obtained from these previous studies can contribute to the better understanding of the biological processes involved in the related to feed efficiency variation. Thus, the study of molecular metabolism regulating FE in slow-growing chicken is needed.

Table 2.3 Summary of gene expression involve in feed efficiency in chicken.

Detail	Traits	Technique	Target	Results	References
Isfahan native chicken vs Broiler	RFI	RNA sequencing	Liver	63 genes were down regulated in the local breed. The altered metabolic processes i.e., carboxylic acid metabolic process and response to stress related to FE	Izadnia et al. (2019)
Chinese local breed (Tianlu Black chicken pure line)	RFI, FCR	SNPs	Blood	55 SNPs in the CCKAR and 4 SNPs of these resulted in amino acid mutations. G1290A was significantly associated with FI and EFI.	Yi et al. (2018)
Broiler (47 days) (High-FE, Low-FE)	RFI, FCR	RNA sequencing	Breast muscle	1,059 DEGs were identified. Up-regulated genes related to remodeling, inflammatory response and free radical scavenging was found in high-FE broilers.	Zhou et al. (2015a)
Broiler (47 days) (High-FE, Low-FE)	RFI, FCR	RNA sequencing	Abdominal fat	286 DEGs were identified. Low-FE chickens had high expression of lipid synthesis genes and lower in triglyceride hydrolysis and cholesterol transport genes.	Zhuo et al. (2015b)

FE = feed efficiency, FCR = feed conversion ratio, RFI = residual feed intake, SNPs = single nucleotide polymorphism, DEGs = differential abundance genes

Table 2.3 Summary of gene expression involve in feed efficiency in chicken (continued).

Detail	Traits	Technique	Target	Results	References
Male broiler (7 weeks) (High-FE ,Low-FE)	FE (g gain: g feed)	Microarray: 44K array, qPCR	Breast muscle	Cytoskeletal architecture (e.g., actin-myosin filaments), fatty acid oxidation, growth factors were upregulated in Low-FE line and signal transduction pathways stimulating energy production were upregulated in high FE line	Bottje et al. (2012)
Male broiler (8 and 10 weeks) (High-FE, Low-FE)	FE (g gain: g feed)	Microarray :44K array, qPCR	Breast muscle	7 genes in the high-FE were down regulated which associated with muscle fiber development, muscle function and cytoskeletal organization	Kong et al. (2011)

FE = feed efficiency, FCR = feed conversion ratio, RFI = residual feed intake, SNPs = single nucleotide polymorphism, DEGs = differential abundance genes

2.5 Impact of feed efficiency on meat quality parameters

As meat quality traits are increasing their market relevance and impacting the quality of final products and consumer perception, becoming one of the most important factors to be considered by the poultry industry (Le Bihan-Duval et al., 2008). Le Bihan-Duval et al. (2008) suggested that the selection to improve FCR may cause unfavorable meat, for example, a greater or lesser degree for L*, drip loss (DL), thawing loss (TL) and shear force (SF) in broilers. Moreover, the study of Wen et al. (2017) demonstrated that the breast muscle of the slow-growing breed (Partridge Shank broilers) had a lower cooking loss than that of the fast-growing breed (Arbor Acres broilers). From table 2.4 showing the correlation between feed efficiency trait and meat quality traits, indicating that the selection of RFI or FCR would influence the variation in meat quality.

Weng et al. (2022) suggested that muscle fiber characteristics between chicken with different FE play a key role regulating meat quality variation. The different in proportion of oxidative fibers and the size of muscular fibers could lead to different meat quality trait (Hwang et al., 2010; Kim et al., 2013). A large proportion of oxidative fibers can positively affect the conversion of muscle to meat, since oxidative fibers showed slower aerobic metabolism, which delays the postmortem metabolic rate (Lefaucheur, 2010). This indicates that there was great meat quality variation among different breeds of chicken. However, little is known the relationship between FE traits and physicochemical characteristics, flavor-related compounds, and biomolecules in the thigh meat of slow-growing chicken. Thus, more efforts are needed to fulfill this gap.

Table 2.4 Correlation between feed efficiency and meat quality in chicken.

Breeds	[1]Traits	Correlations	[2]Results	References
Korat chicken	FCR	Phenotypic	pHu (*r* = 0.1) WHC (*r* = 0.3) DL (*r* = -0.3)	Poompramun et al. (2022)
Chinese slow-growing breed	FCR	Phenotypic	IMF (*r* = 0.15) BWG (*r* = -0.73)	Wen et al. (2018)
	RFI	Phenotypic	IMF (*r* = 0.07) BWG (*r* = 0.07)	
Broilers	FCR	Genetic	pHu (*r* = 0.33) DL (*r* = -0.49) SF (*r* = -0.5)	Paiva et al. (2018)
Label Rouge	FCR	Genetic	Breast yield (*r* = -0.00) Leg yield (*r* = -0.70) Abdominal fat yield (*r* = 0.44)	N'Dri et al. (2016)

[1]FCR: feed conversion ratio, RFI: residual feed intake.

[2] pHu: ultimate pH, WHC: water holding capacity, DL: drip loss, IMF: intramuscular fat, BWG: bodyweight gain, SF: shear force.

2.5.1 pH

In poultry, a normal pH ranges from 5.3 to 5.7 after rigor mortis (Chauhan and England, 2018) and is related to desirable characteristics in meat quality, such as color, shear force, drip loss, intramuscular fat, and water holding capacity (WHC) (Le Bihan-Duval et al., 2008; El Rammouz et al., 2004). A higher and lower pH were previously reported to be linked to the occurrence of Pale Soft Exudative (PSE) and Dark Firm Dry (DFD) in meat, respectively (Lesiów and Kijowski, 2003). A previous transcriptomic study revealed that the variation in muscle pH might be involved with regulations related to carbohydrate, energetic pathway, and muscle remodeling (Beauclercq et al., 2017). The increased activity of glycolysis/ gluconeogenesis pathways observed in the pHu line's breast muscle is directly related to excess energy stored as glycogen and ATP in the muscle. In contrast to the pHu+ muscles, many genes were involved in catabolic and muscle regeneration processes, as well as in response to oxidative stress, indicating that a lack of energy was stored in the muscle (Beauclercq et al., 2016). Moreover, some studies have demonstrated the chickens selected to have a more acidic meat (pHu-) tend to be more efficient than those selected to have a less acidic meat (pHu+), with a higher body weight and better feed efficiency (Berger et al., 2022). This can be indicated that selecting for better feed efficiency can have an impact on pH value in the meat.

2.5.2 Water holding capacity (WHC)

WHC is defined as the ability of fresh meat to hold its moisture during production, processing, and storage, which can be determined by drip loss, cooking loss, and storage loss tests (Huang et al., 2020). WHC determined consumer acceptability and eating quality due to water loss is economically undesirable because it influences meat texture such as juiciness (Herrero, 2008). Lean muscle contains approximately 75% water. The majority of water in muscle is held within the structure of the muscle itself, between muscle cells and between muscle bundles, either within the myofibrils by surface tension or capillary action, which is defined as a "free water". Moreover, water is a dipolar molecule and can be attached to the hydrophilic groups of amino acids in muscle proteins, which is called "chemically bound water" (Huff-Lonergan and M. Lonergan, 2005).

Numerous factors such as pH, hydrolysis, and proteolysis have the potential to greatly influence WHC (Huff-Lonergan and M. Lonergan, 2005). The ability of muscle proteins to bind water decreases as their pH decreases, resulting in poor meat quality. Some studies have also revealed a poor WHC, higher cooking loss and drip loss of the meat of broiler chickens than in Thai native chicken (Jaturasitha et al., 2008) and indigenous Italian Padovana chicken (De Marchi et al., 2005). These results suggested that genetic factor plays a key role in determining variation in WHC in chicken meat. Supporting with previous report of Zuo et al. (2022), showed that metabolites related to energy metabolism and purine metabolism play a key role in influencing the quality of meat. Thus, WHC is one of important indicators for evaluating meat quality.

2.5.3 Nucleotides content

Nucleotides and its derivatives in raw meat such as inosine-5'monophosphate (IMP), guanosine 5'-monophosphate (GMP), adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine (Ino) and hypoxanthine (Hx). IMP and GMP are important meat flavor precursors which closely related to umami taste. IMP is produced by three pathways, de novo synthesis, salvage pathway and IMP transformation (Wegelin et al., 1996). It is well known that chicken meat contains significant amounts of IMP as ATP degrading compound through the enzymatic hydrolysis (Kavitha and Modi, 2007). This compound is serially converted into inosine, ribose and hypoxanthine in the meat.

It has been reported that the content of IMP and GMP broiler chicken meat was higher than Korat chicken meat and Thai native chicken, while a higher content of inosine was observed in Thai native chicken (Katemala et al., 2022). In contrast to the previous study, which reported that Korean native chicken showed higher IMP content compared to broiler chicken (Ahn and Park, 2002). The possibly reason may be due to Korat chicken and native chicken have a higher rate of ATP depletion (Vani et al., 2006). Moreover, the variation in muscle fiber composition between chicken breeds determined the nucleotide content in the meat. Jaturasitha et al. (2016) reported that native chicken muscle contains more oxidative fibers (type I fibers) than domesticated chickens. In rat skeletal muscle, type I muscle fiber showed over-activation of 5′-nucleotidase, which catalyzed the degradation of IMP to

inosine compared to type II muscle fiber (Tullson and Terjung, 1999) This could be hypothesized that the genetic factors may partly explain the nucleotides variations among breeds.

2.5.4 Fourier -transform IR spectroscopy (FT-IR)

Fourier transform infrared (FT-IR) spectroscopy is known as one of the vibrational spectroscopic techniques, used to investigate the chemical composition of each biological sample, which provides a unique IR fingerprint (signature) due to the stretching and bending vibrations of chemical bonds or functional groups that exist in its proteins, fatty acids, carbohydrates, and nucleic acids. Specific wavelengths are absorbed during IR radiation is passed through a sample, causing changes in the chemical bonds in the material associated with vibrations such as stretching, contracting, and bending. Using this method, the resulting peaks will be gained from IR-spectra, representing the vibration of the functional group in the biomolecule's composition in the samples such as protein, fatty acids, nucleic acid, and carbohydrates (Davis and Mauer, 2010; Santos et al., 2015). The band assignment for the biological sample identification is reported in the Table 2.5.

In addition, the advantages and disadvantages of FT-IR method has been reported by Davis and Mauer (2010), 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.

Table 2.5 The band assignment for the biological sample identification (Santos et al., 2015; Davis and Mauer, 2010).

Wave number (cm^{-1})	Molecular vibration of function groups	Functional groups mark	Biomolecule contributor
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
2850	C-H symmetric stretching of >CH2	C-H	Fatty acids
1740	>C=O stretching	C=O	Lipid esters
1715	>C=O stretching of ester	C=O	Nucleic acids and carbonic acids
1695-1675 1600-1690	Amide I band	C=O	Component of proteins
1655	Amide I	C=O	α-helical structure of proteins
1637	Amide I	C=O	β-pleated sheet structure 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	Lipid proteins
1415	C-O-H in-plane bending	C-O-H	Carbohydrates, DNA/RNA, backbone, proteins

Table 2.5 (Continued).

Wave number (cm^{-1})	Molecular vibration of function groups	Functional groups mark	Biomolecule contributor
1400	C=O symmetric stretching of COO- group	C=O	Amino acids, fatty acids
13110-1240 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
720 900-600	C-H rocking of >CH2	C-H	Fatty acids, proteins "Fingerprint region"

2.6 Introduction to the proteomics approach

High-throughput "-omics" technologies (e.g., genomics, transcriptomics, proteomics, metabolomics, metagenomics), are being developed in the post-genomic era, allowing for precisely identifying of functional candidate gene associated with the metabolic pathways affecting feed efficiency (Cánovas et al., 2017). The identification of gene markers controlling the regulation of feed efficiency will be an important data for genetic selection in chicken breeding industry in the next future. However, studying of the genetic background using only genomic and transcriptomic data alone might be not sufficient because it has been demonstrated that mRNA level and protein do not often directly correlate, and the biological complexity is provided by alternate mRNA splicing followed by co- and posttranslational modifications (Burgess et al., 2004). Therefore, this is the reason why we need to get a better understanding of gene functioning at all levels. According to Te pas et al. (2011) reported that the breeding industry is more focused on producing the best genotype, due to production traits are influenced by both genetic and environmental factors, and genetic markers may not be

insufficient to fulfill the demand. Studying in "proteomics, which is defined as the global set of proteins and all of their post-translation modifications expressed in a cell, tissue, or organ at a specific time under specific conditions, could be useful in filling gaps between chicken genomics and phenotypes.

Regarding to the table 2.6 showing the evidence that most studies have analyzed the proteome change impact on muscle growth and development, stress response, but feed efficiency trait. For example, key proteins related to the glucose-pyruvate-tricarboxylic acid (TCA)-oxidative phosphorylation energy metabolism signaling pathway was found to be differ between high- and low-FE pigs (Fu et al., 2017). In addition, Kong et al. (2016) reported mitochondrial dysfunction and oxidative phosphorylation were the main processes regulating feed efficiency. Thus, this indicated that knowing the biological mechanism regulating feed efficiency reveals the possibility of monitoring and modulating the trait. As a result, using a proteomic approach to discover candidate protein biomarkers is necessary for developing breeding programs for selecting more efficient chicken.

From mentioned above, however, the proteomic study on feed efficiency trait in slow-growing chicken remains largely unknown, with most studies focusing only on protein expression in breast meat rather than in other tissues. Since FCR is a ratio measuring of the efficiency with which the bodies of chicken convert feed into the desired output, therefore, it means that not only the chicken meat, but also the chickens' intestines involving in nutrients digestion and absorption system of chicken may play a key role in regulating FE.

Thus, the objective of our study was to characterize and compare the proteomic profiles of KR with high- and low-FCR in three different tissues, breast, thigh meat and small intestine using quantitative proteomic technology by high-resolution label-free liquid chromatography-mass spectrometry (LC-MS). Subsequently, bioinformatic analysis (e.g., functional annotation and pathway analysis) will be used to identify the functional role of key proteins that play a crucial role regulating FE trait. New information concerning the key molecular pathways regulating FE can be applied in selection programs to improve the efficiency of poultry production.

Table 2.6 Summary of proteomic studies in chicken.

Details	Traits	Samples	Proteomic results	References
Female Xinghua chickens	Embryonic development	Leg muscle at embryonic age (E) 11, 16 and 1-day post hatch	·19 up- and 32 down-regulated proteins in E11 vs. E16 ·228 up- and 227 down-regulated proteins in E11 vs. D1 ·13 up- and 5-down-regulated protein in E16 vs. D1	Ouyang et al. (2017)
40 female Beijing-You chickens	Muscle development	Pectoralis muscle was obtained from 1,59,98,140 day	Fast skeletal muscle troponin T isoform, aldehyde dehydrogenase 1A1 and apolipoprotein A1	Liu et al. (2016)
Pedigree male (PedM) broilers	Feed efficiency	Breast muscle	152 differential proteins, Mitochondrial dysfunction, and oxidative phosphorylation up-regulated pathways in high FE group	Kong et al. (2016)
Ross 308 broilers	Stress and postmortem aging	Pectoralis superficialis	Proteome change related to time in transit KPYK, G3P, TPIS, HSP, THOP, HSPB1	Zanetti et al. (2013)

Table 2.6 (Continued.)

Details	Traits	Samples	Proteomic results	References
Korean native chicken & broilers	Meat flavor	Breast, thigh muscle	PGM1, MyHC, HSP 27, Enzyme Q, Glyoxylase 1, DNA Mtase 3,	·Liu et al. (2012)
Broilers	Grow rate, WHC	Breast muscle	Creatine kinase, pyruvate kinase, triosephosphate isomerase, ubiquitin, heat shock protein, myosin heavy chain, actin,	Phongpa-Ngan et al. (2011)
100 Mixed sex Thai indigenous chickens & Broilers	Meat quality (tenderness)	Breast muscle	3 proteins were identified including PKM2, TPI, PGAM1	Mekchay (2010)
20 Mixed sex Thai indigenous chickens	Muscle development	Pectoralis muscles	protein spots were significantly associated with chicken age included PGAM1, APOA1, TPI1, HAP25	Teltathum et al. (2009)
Male layer chickens	Muscle development	Pectoralis muscle	· α-enolase predominates in immature tissue · β-enolase is predominant in mature muscle	Doherty et al. (2004)

WHC = water holding capacity

2.7 Bioinformatic analysis and applications

The definitions of bioinformatics proposed by the US National Institutes of Health (Huerta et al., 2000) and the report of Burgess et al. (2004) have defined bioinformatic and computational biology as follows;

Bioinformatics: research, development, or application of computational tools and approaches for expanding the use of biological, medical, behavioral or health data, including those to acquire, store, organize, archive, analyze, or visualize such data.

Computational biology: the development and application of data-analytical and theoretical methods, mathematical modeling, and computational simulation techniques in the study of biological, behavioral, and social systems

Nowadays, a high-throughput technology such as sequencing and gene/protein profiling techniques has been used to transform biological research by enabling comprehensive monitoring of a biological system. a list of differentially expressed protein that have roles in a given phenotype give us a new challenge, by extracting meaning from a long list of differentially expressed proteins.

Form the report of Katri, Sirota and Butte (2012) explained one approach to this challenge in bioinformatics is that pathway analysis, the first choice for gaining insight into the underlying biology of differentially expressed proteins, as it reduces complexity. Two reasons appealing for analyzing high-throughput molecular measurements at the functional level, First, grouping thousands of proteins by the pathways they are involved in reduces the complexity to just several hundred pathways for the experiment. Second, identifying active pathways that differ between two conditions can have more explanatory power than a simple list of different proteins.

2.7.1 Function enrichment and pathway analytic approaches

Functional annotation has been applied to the analysis of Gene ontology (GO) terms when trying to bring proteins from a list into some biologically meaningful context. This approach composes of biological process, molecular function, and cellular component. The DAVID program is a widely used web-based application focusing on GO classification (Werner, 2008)

Moreover, in pathways terms is another approach to carry the analysis deeper into biology, going to the molecular level. Pathways focus on physical and

functional interactions between protein rather than a list of individual protein by identifying active pathways that differ between two conditions (Werner. 2008) and Kyoto Encyclopedia of Genes and Genomes (KEGG) is wildly used software for pathway analysis.

From the mentioned above paragraph, with the information that we will gain for high-throughput technology such as protein profiling and pathway analysis would be helpful for researchers who need a deeper understanding in the biological mechanism in all levels of gene functioning. Consequently, this will be useful to get a precision breeding.

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CHAPTER III

COMPARATIVE PROTEOMICS REVEALED DUODENAL METABOLIC FUNCTION ASSOCIATED WITH FEED EFFICIENCY IN SLOW-GROWING CHICKEN

3.1 Abstract

The Korat chicken (KR), developed in Thailand, is a slow-growing breed developed as an alternative breed for Thai chicken producers. The growing interest in slow-growing chicken meat, due to its unique taste, distinct texture, health benefits, and higher broiler welfare have led to higher market demand for KR. However, its low feed efficiency (FE) has a significant negative impact on farm profitability. Understanding the molecular mechanism regulating FE allows for designing a suitable selection program and contributing to breeding more efficient chicken for poultry production. Thus, the objective of our study was to investigate the proteome differences and possible pathways associated with FE in male KR using a label-free quantitative proteomic approach. Seventy-five KR males were individually evaluated for FE, and duodenum samples from 6 animals (3 high-FE and 3 low-FE chickens) were collected at 10 wk of age for differential abundant proteins (DAPs), protein networks, functional enrichment, and pathway analyses. In this study, we found 40 DAPs significantly associated with FE pathways, including glycolysis/gluconeogenesis, peroxisome, oxidative phosphorylation, tight junction, and cysteine and methionine metabolism. Thus, variations in observed DAPs or genes related to DAPs could be interesting biomarker candidates for selection for higher feed utilization efficiency in chicken.

Key words: Korat chicken, feed efficiency, slow-growing chicken, label-free proteomics

3.2 Introduction

Consumers are increasingly interested in meat quality, safety, and animal welfare. These trends favor slow-growing chickens due to the breeds' meat characteristics, including unique taste, firmer texture, higher nutritional value, and welfare compared to fast-growing commercial breeds (Lusk, 2018). In Thailand, Korat chicken (KR), was developed as an alternative meat-type chicken for producers. Korat chicken is a crossbreed between the Thai indigenous Leung Hang Khao chicken and the Suranaree University of Technology (SUT) synthetic line. Despite its good meat characteristics, the growth rates and feed efficiency (FE) of KR are low, causing low profits (Hang et al., 2018). Thus, to increase its competitiveness in the Thai poultry production market and to offer an efficient alternative breed for small- to moderate-sized Thai farms, improving FE is the most important breeding goal in KR chicken.

Feed efficiency is most often measured as a feed conversion ratio (FCR), that is, how many kgs of feed are needed to produce 1 kg of body mass. The heritability of FCR in chickens is moderate, allowing for efficient selection. For example, the estimated heritability for Arkansas broilers was 0.49 (Aggrey et al., 2010) and the estimate for the commercial slow-growing meat-type chicken line was 0.33 (N'Dri et al., 2006). Although improving FCR is possible through selection, it is important to understand the biological basis of FCR given its complexity. Feed efficiency depends on feed intake, energy homeostasis, intestinal structure, and many physiological processes related to the utilization of feed, including intestinal nutrient digestion, absorption, the integrity of the intestinal epithelium, and translocation of intestinal antigens (Richards and Proszkowiec-Weglarz, 2007; Choct, 2009; Nain et al., 2012). Previous studies have shown that high-FE chickens have longer gastrointestinal tracts (Kadhim et al., 2010; Krás et al., 2013; Mabelebele et al., 2014), higher nutrient digestibility (Rougiere et al., 2009; De Verdal et al., 2010), and larger duodenal absorptive villi surface (Nain et al., 2012) than low-FE chicken. The duodenum is a complex organ with an important role in FE, as it regulates the feed digestion process and energy homeostasis (Recoules et al., 2019).

Genome-wide association studies have revealed genomic regions and candidate genes associated with FCR (Mebratie et al., 2019). In addition, transcriptomic studies have revealed pathways related to FE through the digestive function of the

duodenum in meat-type chicken (Aggrey et al., 2014; Lee et al., 2015). As the relationship between gene expression levels and their corresponding protein abundance is indirect and the physiological processes are mainly controlled by protein levels (Burgess, 2004), the knowledge from genomic and transcriptomic studies may not be enough to explain the genetic basis of FE. Therefore, proteomic analysis may provide additional insight into the functional mechanisms underlying FE (Kong et al., 2016a; Fu et al., 2017; Fonseca et al., 2019). Currently, little is known about the association between FE traits and proteomics in the small intestinal tissues of chicken, especially in the duodenal part.

A previous study of the pig intestinal proteome revealed important pathways associated with small intestinal structures and movements, including the regulation of actin cytoskeleton, focal adhesion, adherens junction, tight junction, and vascular smooth muscle contraction (Wu et al., 2020). The results suggested that these major physiological processes play a key role in maintaining the integrity of the intestinal epithelium, which is important for digestion and absorption capacity. Therefore, we hypothesized that changes in protein function related to physiological and biological processes in the duodenum may contribute to the FE of chicken.

The objective of our study was to characterize and compare the duodenal proteomic profiles of KR with high- and low-FE using quantitative proteomic technology by high-resolution label-free liquid chromatography-mass spectrometry (LC-MS). New information concerning the key molecular pathways regulating FE can be applied in selection programs to improve the efficiency of poultry production.

3.3 Materials and methods

3.3.1 Experiment chickens and phenotypic data collection

The birds used in this study belonged to the KR breed. Each KR generation was formed by crossing Leung Hang Khao males and SUT synthetic line females. To produce the set of birds used in this study, 5th generation KR parental birds with the highest body weight were mated together, and 5th generation KR parental birds with the lowest body weight were mated together. At hatching, the birds were sexed using the vent sexing method, wing banded, and vaccinated against Marek's disease. Thereafter, they were vaccinated following the recommendation of

the Department of Livestock development, Thailand. Seventy-five 1-day-old male KR were individually housed in cages (63x125x63 cm) covered with rice hulls. All birds were given access to feed and water ad libitum in similar environmental conditions. The same diet was provided to all birds throughout the experiment period using a starter diet (21% protein) for birds 0 to 3 wk of age, a grower diet (19% protein) for birds 4 to 6 wk of age, and a finisher diet (17% protein) for birds 7 to 10 weeks of age. A watering line was supplied across the compartment and attached by nipple drinkers to each cage. Total feed intake and body weight gain from 1 to 10 weeks were measured to calculate FCR:

$$FCR = \frac{FI}{BWG},$$

where FI represents the total feed intake from wk 1 to wk 10 (g) and BWG represents the body weight at wk 10 minus the body weight at wk 1 (g).

At 10 wk of age, the chicken was ranked based on their FCR values. Three chickens with the highest FCR (FCR = 3.33, 3.34, and 3.36) and 3 chickens with the lowest FCR (FCR = 1.83, 1.98, and 1.99) were selected as a low-FE and high-FE groups, respectively, and as a group for the proteomic analysis (3 + 3 biological replicates).

3.3.2 Duodenal sample collection

At the age of 10 wk, all birds were slaughtered with electrical stunning and exsanguination after eight hours of fasting. The intestinal tract was immediately removed, and the whole duodenum was collected and stored in liquid nitrogen at -80°C. During the procedure, dissecting instruments were cleaned with 70% ethanol after each individual bird to prevent cross contamination.

3.3.3 Protein extraction

The frozen duodenum samples were freeze-dried, crushed to a fine powder, and lysed in 50 mM ammonium bicarbonate buffer (AMBIC) containing 8 M urea (Sigma-Aldrich, St. Louis, MO). The lysed proteins were sonicated on ice and isolated by centrifugation at 20,000 g for 10 min at 4°C. Protein samples were diluted with 50 mM ammonium bicarbonate buffer to a final concentration of 1.5 M urea. Protein concentration was measured using the Pierce BCA Protein Assay kit (Thermo

Fisher Scientific, Waltham, MA). Then, 100 mg of proteins from each sample were transferred to a 1.5-mL tube. Finally, the protein was reduced for 20 min at 50°C to 60°C with a final concentration of 5 mM dithiothreitol (DTT) and then alkylated for 20 min at room temperature in the darkness with a final concentration of 15 mM iodoacetamide (IAA).

3.3.4 Mass spectrometry of the protein samples

Protein samples were digested with 2 mg trypsin (Promega Corporation, Madison, WI) overnight at 37°C. Mass spectrometry analysis was carried out in a Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA) at the Proteomics Unit core facility, University of Helsinki, Finland. The peptides were separated on a C18 reverse-phase column on an 80min gradient, and the analysis was carried out using higher-energy collisional dissociation (HCD) for mass fragmentation and data-dependent acquisition (DDA) mode. One technical replicate of each 6 samples was combined to perform the database search. The raw proteomic data sets in the current study are available on the ProteomeXchange Consortium via the PRIDE (https://www.ebi.ac.uk/ pride/) partner repository, with the data set identifier PXD027317.

3.3.5 Protein identification analysis

The raw data from Orbitrap mass spectrometry were imported into MaxQuant software version 1.6.5.0 (Cox and Mann, 2008) for peptide matching to MS/MS spectra. Resulting spectra were identified against the Uniport database of Gallus gallus reference proteome (34,925 entries, downloaded from https://www.uniprot.org, January 2019 version). The parameters for the protein identification were trypsin specificity; two missed cleavages and methythio (C) was selected as a fixed modification, and oxidation (M) and acetyl (protein N-term) as a variable modification. The initial precursor (MS) mass tolerance was set to 20 ppm in the first search and 6 ppm in the main search. Additionally, fragment (MS/MS) mass deviation was set to 20 ppm and both peptide and protein false discovery rates (FDR) were set to 1%. The MaxQuant label-free quantification (LFQ) algorithm was used for quantification (minimum ratio count = 2).

3.3.6 Differential proteomic analysis

The LFQ intensity values generated by MaxQuant (Cox and Mann, 2008) were used in Perseus software version 1.6.5.0 (Tyanova et al., 2016) for statistical analyses and data visualization. Prior to the analysis, we removed proteins identified by post-translation modification, contaminant proteins, or hits the reverse sequence. Only proteins occurring in 2 out of 3 biological replicates in both experiment groups were kept. Label-free quantification intensity values were transformed to a logarithmic scale with a base of 2. Missing values were imputed from a normal distribution (width: 0.3, down shift: 1.8). Student's t test was used for comparison between the high- and low-FE groups. Proteins with a P-value <0.05 were considered DAPs. Hierarchical clustering was performed with DAPs after Z-score normalization.

The visualization of the differences and similarities of the proteomic profiles and DAPs between the high- and low-FE groups was constructed using a principal component analysis (PCA) with the ggplot2 (Wickham, 2009) and ggfortify (Tang et al., 2016) packages in R version 3.5.2 (R Core Team, 2020).

3.3.7 Bioinformatics analysis of differentially abundant proteins

Gene ontology (GO) enrichment, networks of protein-protein interaction (PPI), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using the STRING platform (version 10, http://string-db.org) against the Gallus gallus database and considering a medium confidence score of 0.4 for interaction (Szklarczyk et al., 2015). The GO enriched proteins and KEGG pathways were considered enriched with a P value <0.05, correcting by FDR with Benjamini-Hochberg method (FDR < 0.05).

3.4 Results and discussion

3.4.1 Performance and feed efficiency parameters

The performances of two KR chicken groups are illustrated in Table 3.1. As expected, the difference in FCR between the high-FE (1.93±0.05) and the low-FE (3.34±0.01) groups was highly significant (P value < 0.01). Moreover, the body weight gain of the high-FE group was significantly higher than that of the low-FE group (P value = 0.01), while the differences in feed intake were not significant (P value =

0.080). Thus, the differences in FCR could be mainly explained by the differences in functions related to weight gain.

Table 3.1 Growth performance of the high-FE and low-FE groups from 1 to 10 weeks of age (Mean±standard error)

Traits	High-FE (n=3)	Low-FE (n=3)	*P*-value[1]
FI (g)	3173.07±209.25	3807.38±168.30	0.080
BWG (g)	1638.08±84.60	1138.93±49.30	0.012
FCR	1.93±0.05	3.34±0.01	<0.01

[1] Comparison between High-FE and Low-FE groups by a t-test

Abbreviations: FI, total feed intake from 1 week to 10 weeks; BWG, body weight gain; FCR, feed conversion ratio

3.4.2 Duodenal proteome identification

A total of 1,013 proteins were initially identified by high throughput proteomics analysis after eliminating any unnecessary or incorrect protein identifications. Summary information about mass spectrometry analysis can be found in the Supplementary Table 3.1. Out of the 1,013 identified proteins, 567 proteins were common for both high- and low-FE groups, constituting 56% of the total proteins identified, while 229 (23%) proteins were present only in the high-FE group and 167 (16%) proteins in the low-FE group (Figure 3.1).

The results suggest that the variation in FE is reflected at the type and level of the duodenal proteome. In contrast to a previous proteomic study, which characterized altered mitochondrial proteins on the skeletal tissue of male broiler exhibiting high-FE and low-FE phenotypes (Kong et al., 2016a), our study is the first to investigate the alteration in the duodenal tissue of a slow-growing chicken using a label-free method, which can provide comprehensive information on FE in chicken.

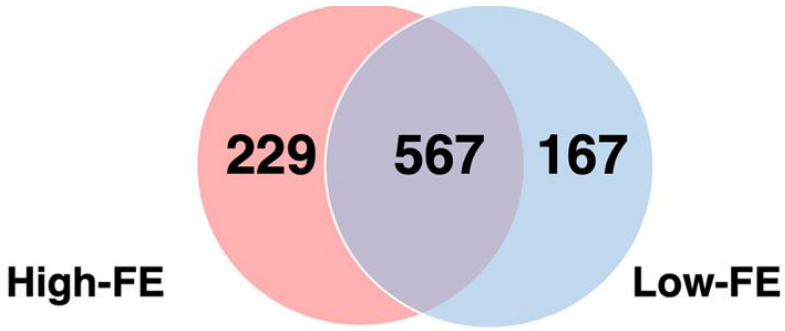

Figure 3.1 A Venn Diagram representing the number of identified proteins in the duodenal tissue of the Korat chicken that were unique for the high-FE (left) or the low-FE (right) groups or were common for both groups (center).

3.4.3 Differential proteomic analysis between KR chicken with high- and low-FE

Out of the 567 common proteins, 355 proteins were present in 2 out of 3 biological samples in both groups (Supplementary Table 3.2). A PCA plot was conducted to illustrate the similarities and differences in the proteomic profiles of the high- and low-FE groups. The result revealed that principal component 1 (PC1) explained over 43% of the variance in proteomic abundance. Moreover, the low-FE samples were more closely clustered together, whereas the high-FE samples were scattered, indicating more natural biological variation in protein abundance in the high-FE group than in the low-FE group (Supplementary Figure 3.1).

The differential protein abundance analysis revealed 40 DAPs that had significantly different abundances between the high- and low-FE groups based on P value < 0.05 (Table 3.2). The hierarchical clustering of the DAPs is illustrated in Figure 3.2, which showed clear discrimination between the DAP clusters of the 2 FE groups. Within the 40 DAPs, 14 proteins had high abundance in the high-FE group and 26 proteins had high abundance in the low-FE group. Moreover, despite the high level of natural variation in the high-FE group, the PCA plot of the DAPs also showed a clear separation between the two groups, providing evidence that these DAPs are appropriate for group separation (Supplementary Figure 3.2).

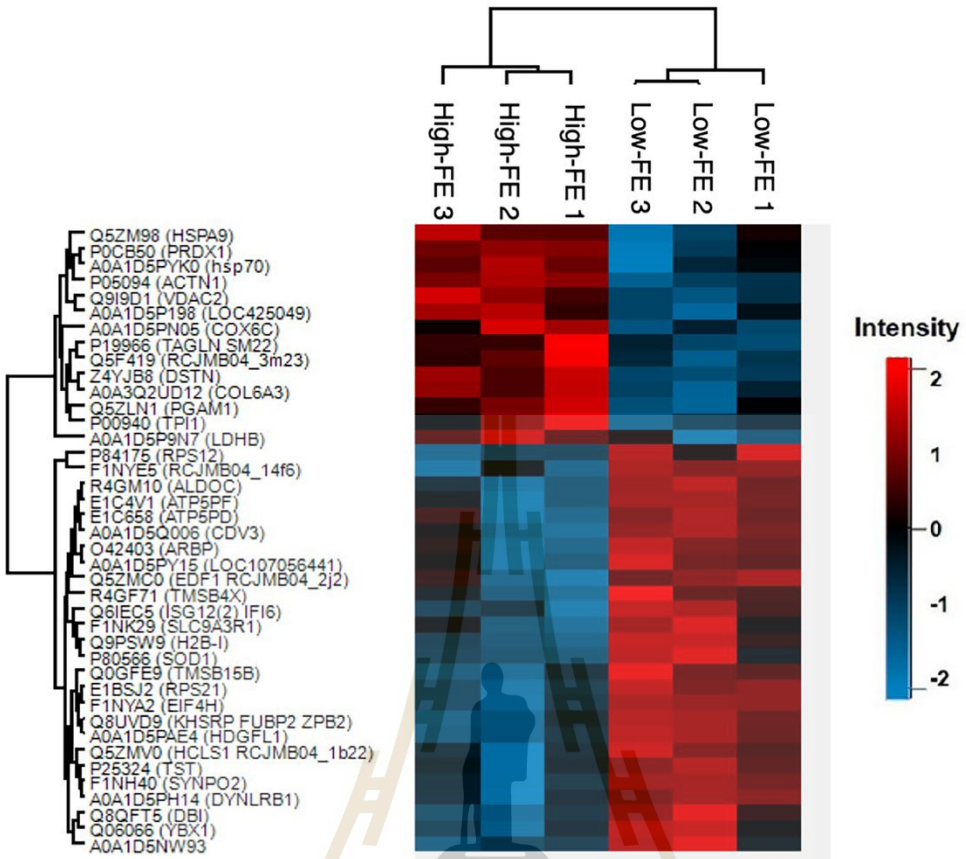

Figure 3.2 Dendrogram representing the hierarchical clustering of the 40 DAPs in the high-FE and in the low-FE groups. Abbreviation: DAPs, differentially abundant proteins.

Table 3.2 List of 40 DAPs between the high- and low-FE groups.

Uniprot ID[1]	Protein name	Gene name	*P*-value	FC
Q5ZM98	Stress-70 protein	HSPA9	0.043	-0.55
P0CB50	Peroxiredoxin-1	PRDX1	0.025	-0.69
A0A1D5PYK0	Heat shock cognate 71 kDa protein	HSPA8	0.038	-0.78
P05094	Alpha-actinin-1	ACTN1	0.001	-0.66
Q9I9D1	Voltage-dependent anion channel	VDAC2	0.004	-0.59
A0A1D5P198	Tubulin alpha chain	LOC425049; TUBA3E	0.018	-0.77
A0A1D5PN05	Cytochrome-c oxidase activity	COX6C	0.021	-0.38
P19966	Transgelin	TAGLN	0.035	-0.42
Q5F419	VAMP-associated protein	RCJMB04_3m23; VAPA	0.023	-0.37
Z4YJB8	Destrin	DSTN	0.002	-0.56
A0A3Q2UD12	Collagen alpha-3(VI) chain	COL6A3	0.007	-0.86
Q5ZLN1	Phosphoglycerate mutase 1	PGAM1	0.030	-0.38
P00940	Triosephosphate isomerase 1	TPI1	0.045	-0.55
A0A1D5P9N7	L-lactate dehydrogenase B chain	LDHB	0.043	-0.62
P84175	40S ribosomal protein S12	RPS12	0.017	0.51
F1NYE5	HABP4_PAI-RBP1 domain-containing protein	RCJMB04_14f6	0.011	1.92
R4GM10	Fructose-bisphosphate aldolase C	ALDOC	0.006	0.29
E1C4V1	ATP synthase-coupling factor 6	ATP5PF; ATP5J	0.015	0.68
E1C658	ATP synthase subunit d	ATP5PD; ATP5H	0.048	1.33
A0A1D5Q006	Protein CDV3 homolog	CDV3	0.018	2.26
O42403	Attachment region binding protein	ARBP	0.032	1.08
A0A1D5PY15	Coronin	LOC107056441; CORO1B	0.033	0.61
Q5ZMC0	Endothelial differentiation-related factor 1	EDF1	0.036	0.85
R4GF71	Thymosin beta	TMSB4X	0.033	0.65
Q6IEC5	Putative ISG12(2) protein	ISG12(2) IF16	0.017	1.48

Table 3.2 List of 40 DAPs between the high- and low-FE groups (Continued).

Uniprot ID[1]	Protein name	Gene name	*P*-value	FC
F1NK29	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	SLC9A3R1	0.048	1.00
Q9PSW9	Histone H2B-I	H2B-I	0.012	0.53
P80566	Superoxide dismutase [Cu-Zn]	SOD1	0.032	0.45
Q0GFE9	Thymosin beta	TMSB15B	0.015	0.64
P84175	40S ribosomal protein S12	RPS21	0.004	0.50
F1NYA2	RRM domain-containing protein	EIF4H	0.003	0.78
Q8UVD9	Far upstream element-binding protein 2	KHSRP FUBP2 ZPB2	0.006	0.22
A0A1D5PAE4	Heparin binding growth factor	HDGFL1	0.004	1.17
Q5ZMV0	SH3 domain-containing protein	HCLS1	0.035	1.28
P25324	Thiosulfate sulfurtransferase	TST	0.046	0.72
F1NH40	Synaptopodin 2	SYNPO2	0.017	1.60
A0A1D5PH14	Dynein light chain roadblock	DYNLRB1	0.025	0.90
Q8QFT5	Diazepam binding inhibitor	DBI	0.020	0.64
Q06066	Y-box-binding protein 1	YBX1	0.040	0.77
A0A1D5NW93	ATP synthase F1 subunit delta	ATP5PD; ATP5D	0.039	0.43

Abbreviations: DAPs, differentially abundant proteins; FC, fold change.

[1] Protein accession number from the Uniprot database (www.uniprot.org)

3.4.4 Functional enrichment of the DAPs

We conducted functional enrichment analysis to associate the DAPs with their biological processes, molecular functions, and cellular components. The results of the enrichment analysis are presented in Table 3.3. Among the biological processes, the largest number of DAPs (15 out of 40) were related to cellular process (GO:0009987). The other common biological processes were cellular metabolic process (GO:0044237) (10 out of 40 DAPs) and the regulation of cellular process (GO:0050794) (8 out of 40 DAPs). The 3 most common molecular functions were binding (GO:0005488) (13 out of 40 DAPs), protein binding (GO:0005515) (8 out of 40 DAPs), and catalytic activity (GO:0003824) (7 out of 40 DAPs). The largest number of DAPs in the cellular component were related to intracellular part (GO:0044424) (19

out of 40 DAPs), cytoplasm (GO:0005737) (17 out of 40 DAPs), and intracellular organelle (GO:0043229) (12 out of 40 DAPs).

The results indicate that most of the DAPs found between high- and low-FE groups relate to many essential metabolic processes that function in the duodenum. Given that the duodenum is the main organ in the nutrient digestion process, the results also reveal the biochemical and physiological aspects of molecular metabolism regulating FE. In addition, our results support previous findings that several physiological processes, for example, feed intake, feed digestion, metabolism, physical activity, and thermoregulation relate to FE (Herd and Arthur, 2009).

Table 3.3 Significant Gene ontology (GO) terms of DAPs listed in Table 3.1.

GO ID	Description	adj. *P* value[1]	Proteins
Biological process			
GO:0006090	pyruvate metabolic process	3.00E-05	ALDOC, LDHB, PGAM1, TPI1
GO:0051186	cofactor metabolic process	3.00E-05	ALDOC, HSPA9, PGAM1, PRDX1, TPI1
GO:0009987	cellular process	0.0001	ACTN1, ALDOC, DSTN, EDF1, H2B-I, HSPA9, LDHB, PGAM1, PRDX1, RPS12, SLC9A3R1, SOD1, TAGLN, TPI1, YBX1
GO:0005975	carbohydrate metabolic process	0.0003	ALDOC, LDHB, PGAM1, TPI1
GO:0006006	glucose metabolic process	0.0003	ALDOC, PGAM1, TPI1
GO:0006094	gluconeogenesis	0.0003	ALDOC, PGAM1, TPI1
GO:0006096	glycolytic process	0.0003	ALDOC, PGAM1, TPI1
GO:0006754	ATP biosynthetic process	0.0003	ALDOC, PGAM1, TPI1
GO:0006757	ATP generation from ADP	0.0003	ALDOC, PGAM1, TPI1
GO:0009166	nucleotide catabolic process	0.0003	ALDOC, PGAM1, TPI1
GO:0017144	drug metabolic process	0.0003	ALDOC, PGAM1, PRDX1, TPI1

[1]FDR-adjusted *P*-values

Table 3.3 Significant Gene ontology (GO) terms of DAPs listed in Table 3.1 (Continued).

GO ID	Description	adj. *P* value[1]	Proteins
GO:0019359	nicotinamide nucleotide biosynthetic process	0.0003	ALDOC, PGAM1, TPI1
GO:0042866	pyruvate biosynthetic process	0.0003	ALDOC, PGAM1, TPI1
GO:0046496	nicotinamide nucleotide metabolic process	0.0003	ALDOC, PGAM1, TPI1
GO:0009168	purine ribonucleoside monophosphate biosynthetic process	0.0003	ALDOC, PGAM1, TPI1
GO:0019430	removal of superoxide radicals	0.0003	PRDX1, SOD1
GO:0044237	cellular metabolic process	0.0004	ALDOC, EDF1, HSPA9, LDHB, PGAM1, PRDX1, RPS12, SOD1, TPI1, YBX1
GO:0009167	purine ribonucleoside monophosphate metabolic process	0.0008	ALDOC, PGAM1, TPI1
GO:0044248	cellular catabolic process	0.0012	ALDOC, PGAM1, PRDX1, TPI1
GO:0044271	cellular nitrogen compound biosynthetic process	0.0019	ALDOC, EDF1, PGAM1, RPS12, TPI1, YBX1
GO:1901566	organonitrogen compound biosynthetic process	0.0031	ALDOC, PGAM1, RPS12, TPI1
GO:0034654	nucleobase-containing compound biosynthetic process	0.0052	ALDOC, EDF1, PGAM1, TPI1, YBX1
GO:1901576	organic substance biosynthetic process	0.0059	ALDOC, EDF1, PGAM1, RPS12, TPI1, YBX1

[1]FDR-adjusted *P*-values

Table 3.3 Significant Gene ontology (GO) terms of DAPs listed in Table 3.1 (Continued).

GO ID	Description	adj. *P* value[1]	Proteins
GO:0055114	oxidation-reduction process	0.0066	LDHB, PRDX1, SOD1
GO:0050794	regulation of cellular process	0.0077	COL6A3, DSTN, EDF1, HSPA9, PGAM1, PRDX1, SLC9A3R1, YBX1
GO:0007015	actin filament organization	0.0088	ACTN1, DSTN
GO:0051171	regulation of nitrogen compound metabolic process	0.0097	COL6A3, EDF1, PGAM1, PRDX1, SLC9A3R1, YBX1
GO:0080090	regulation of primary metabolic process	0.0101	COL6A3, EDF1, PGAM1, PRDX1, SLC9A3R1, YBX1
GO:0031323	regulation of cellular metabolic process	0.0109	COL6A3, EDF1, PGAM1, PRDX1, SLC9A3R1, YBX1
GO:0060255	regulation of macromolecule metabolic process	0.0109	COL6A3, EDF1, PGAM1, PRDX1, SLC9A3R1, YBX1
GO:0044238	primary metabolic process	0.0186	ALDOC, EDF1, LDHB, PGAM1, RPS12, TPI1, YBX1
GO:0019220	regulation of phosphate metabolic process	0.0211	PGAM1, PRDX1, SLC9A3R1
GO:0071704	organic substance metabolic process	0.0223	ALDOC, EDF1, LDHB, PGAM1, RPS12, TPI1, YBX1
GO:0006139	Nucleobase compound metabolic process	0.0260	ALDOC, EDF1, PGAM1, TPI1, YBX1
GO:0006139	nucleobase-containing compound metabolic process	0.0260	ALDOC, EDF1, PGAM1, TPI1, YBX1
GO:0022607	cellular component assembly	0.0497	ACTN1, H2B-I, HSPA9

[1]FDR-adjusted *P*-values

Table 3.3 Significant Gene ontology (GO) terms of DAPs listed in Table 3.1 (Continued).

GO ID	Description	adj. *P* value[1]	Proteins
Molecular function			
GO:0005488	binding	0.0004	ACTN1, DBI, DSTN, EDF1, H2B-I, HSPA8, HSPA9, PRDX1, SLC9A3R1, SOD1, TAGLN, TST, YBX1
GO:0005515	protein binding	0.0024	ACTN1, DSTN, H2B-I, HSPA8, HSPA9, PRDX1, SLC9A3R1, TAGLN
GO:0051015	actin filament binding	0.0024	ACTN1, DSTN, TAGLN
GO:0016209	antioxidant activity	0.0049	PRDX1, SOD1
GO:0051219	phosphoprotein binding	0.0049	ACTN1, HSPA8
GO:0003824	catalytic activity	0.0066	ALDOC, LDHB, PGAM1, PRDX1, SOD1, TPI1, TST
GO:0016853	isomerase activity	0.0073	PGAM1, TPI1
GO:0016491	oxidoreductase activity	0.0117	LDHB, PRDX1, SOD1
GO:0097159	organic cyclic compound binding	0.0117	DBI, EDF1, H2B-I, HSPA8, HSPA9, TST, YBX1
Cellular component			
GO:1901363	heterocyclic compound binding	0.0117	DBI, EDF1, H2B-I, HSPA8, HSPA9, TST, YBX1
GO:0005737	cytoplasm	7.10E-09	ACTN1, ALDOC, CDV3, DBI, EDF1, HSPA8, HSPA9, LDHB
GO:0005737	cytoplasm	7.10E-09	PGAM1, PRDX1, RPS12, SLC9A3R1, SOD1, TAGLN, TPI1, TST, YBX1
GO:0044424	intracellular part	7.10E-09	ACTN1, ALDOC, CDV3, DBI, DSTN, EDF1, H2B-, HSPA8, HSPA9, LDHB, PGAM1, PRDX1, RPS12, SLC9A3R1, SOD1, TAGLN, TPI1, TST, YBX1

[1]FDR-adjusted *P*-values

Table 3.3 Significant Gene ontology (GO) terms of DAPs listed in Table 3.1 (Continued).

GO ID	Description	adj. *P* value[1]	Proteins
GO:0044444	cytoplasmic part	0.0001	ACTN1, ALDOC, DBI, EDF1, HSPA9, LDHB, PGAM1, RPS12, TPI1, TST
GO:0043229	intracellular organelle	0.0004	ACTN1, DBI, DSTN, EDF1, H2B-I, HSPA8, HSPA9, PRDX1, RPS12, SOD1, TST, YBX1
GO:0005829	cytosol	0.0004	ALDOC, EDF1, LDHB, PGAM1, RPS12, TPI1
GO:0043231	intracellular membrane-bounded organelle	0.0077	DBI, EDF1, H2B-I, HSPA8, HSPA9, PRDX1, SOD1, TST, YBX1
GO:0001726	ruffle	0.0098	ACTN1, SLC9A3R1
GO:0043209	myelin sheath	0.0098	PGAM1, PRDX1
GO:1990904	ribonucleoprotein complex	0.0106	HSPA8, RPS12, YBX1
GO:0032991	protein-containing complex	0.0139	COL6A3, EDF1, H2B-I, HSPA8, RPS12, YBX1
GO:0043232	intracellular non-membrane-bounded organelle	0.0244	ACTN1, DSTN, EDF1, H2B-I, RPS12

[1]FDR-adjusted *P*-values

3.4.5 Protein interaction network and enrichment pathways of DAPs

Analysis of the PPI network revealed nine proteins (H2B-I, ISG12-2, EDF1, DBI, DYNLRB1, SLC9A3R1, HDGF, TUBA3E, CDV3) that had no interaction with other DAPs (Figure 3.3). This may indicate that these proteins may not be biologically relevant or may have independent functions. However, the majority of DAPs interacted with each other and formed clusters, comprising with metabolic enzymes (PGAM1, TPI1, ALDOC, LDHB), cytoskeleton proteins (DSTN, CORO1B, TAGLN), ribosomal proteins (SERBP1, RPS21, RPS1), translational initiation factor (EIF-4H), stress-responsive proteins

(PRDX1, SOD1, HSPA8, HSPA9), and electron transport chain proteins (VDAC2, ATP5J, ATP5D, ATP5H), which interacted closely with VAPA, TST, and YBX1. Further, the small components of interacting proteins were also presented in this network, including ACTN1, TMSB4X, SYNPO2, and COL6A3. Such interactions may indicate that these proteins function cooperatively in FE regulation.

DAPs were further explored based on the KEGG pathway to elucidate the metabolic pathway in which these proteins were involved. Five enriched pathways identified are presented in Table 3.4: glycolysis/gluconeogenesis, peroxisome, oxidative phosphorylation, tight junction, and cysteine and methionine metabolism. Below we have discussed the potential roles of these proteins in functional pathways that could affect FE.

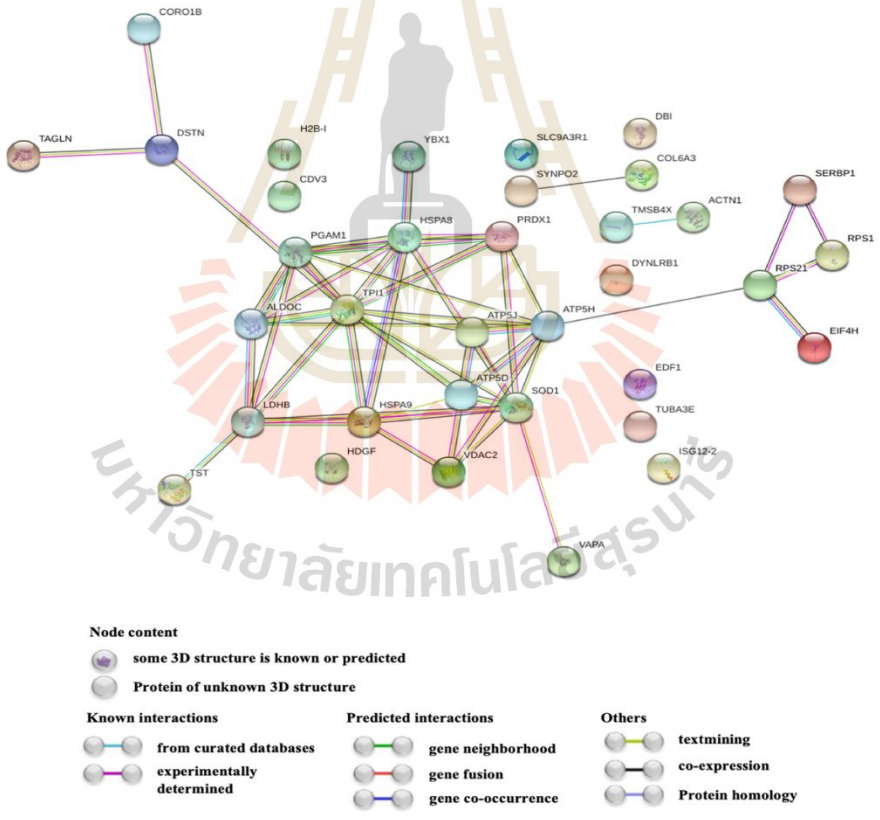

Figure 3.3 Protein-protein interaction network of DAPs. Nodes represent the DAPs identified with the coding gene symbol, colored nodes indicate the query proteins, and lines represent the connections between the proteins. Abbreviation: DAPs, differentially abundant proteins.

Table 3.4 Enriched metabolic pathways of DAPs. High abundant proteins in the high-FE group are marked in bold face.

KEGG ID	Description	adj. *P* value[1]	Proteins
00010	Glycolysis/Gluconeogenesis	0.0002	ALDOC, **LDHB**, **PGAM1**, **TPI1**
04146	Peroxisome	0.048	**PRDX1**, SOD1
00190	Oxidative phosphorylation	0.018	ATP5J, ATP5H, ATP5D
04530	Tight junction	0.018	**ACTN1**, SLC9A3R1, **TUBA3E**
00270	Cysteine and methionine metabolism	0.018	**LDHB**, TST

[1]FDR-adjusted *P*-values

3.4.6 Proteins related to carbohydrate metabolism

The glycolysis/gluconeogenesis pathway is the most enriched term of DAPs. It is well known that glucose catabolism of the intestinal tract is essential for providing energy during the digestion and absorption process (Fändriks, 2017). In glycolysis, glucose breakdown generates 2 molecules of pyruvate, which is the main glycolytic product that can be oxidized to produce ATP for cellular metabolism through the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (Fonseca et al., 2019). Therefore, it was interesting that 3 proteins were among the most abundant in the duodenum of the high-FE group, including triphosphate isomerase (TPI1) and phosphoglycerate mutase 1 (PGAM1), which are glycolytic enzymes that catabolize glucose into pyruvate (Alberts et al., 2002), and lactate dehydrogenase (LDHB), an enzyme that converts lactate to pyruvate (Zhao et al., 2020). These three enzymes are important in the rumen epithelium of inefficient steers (Kong et al., 2016b) and also in the jejunum of low-FCR chicken (Shah et al., 2019). Thus, higher levels of these 3 enzymes in the high-FE group can have a major impact on the pyruvate generation rate, resulting in higher ATP production in the high-FE group compared to the low-FE group. Our result also indicates that high-FE chicken can use more glucose as an oxidative substrate for energy generation in the duodenal epithelium than low-FE chicken.

3.4.7 Proteins related to cysteine and methionine metabolism

Glucogenic amino acids, cysteine, and methionine are the major precursors that can be converted into glucose (Brosnan, 2003). In this study, thiosulfate sulfurtransferase (TST) was a DAP in the low-FE group. Thiosulfate sulfurtransferase is an enzyme involved in sulfide catabolism to sulfite, sulfate, and thiosulfate, which is important for producing cysteine from methionine via the transsulfuration pathway (Kohl et al., 2019). Thus, increased levels of this enzyme may provide more compensatory precursors for gluconeogenesis through amino acid metabolism in the low-FE group to meet their energy demands. In our findings, the abundance of aldolase C (ALDOC) was higher in the low-FE group than in the high-FE, supporting this assumption. ALDOC catalyzes the reversible dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (G3P) to form fructose-1,6-bisphosphate (F-1,6-BP) in the gluconeogenic direction. Our results are consistent with Zhang et al. (2019), who reported that proteins involved in gluconeogenesis and amino acid metabolism in the livers of low-FE heifers were upregulated. Although previous studies have shown that the carbon transaction process between amino acid and carbohydrate metabolism mainly occurs in the liver and muscles of chicken (Abasht et al., 2019; Yang et al., 2020), our study revealed that high-abundant protein related to the metabolic fate of amino acids and glucose can occur in the intestinal tract of slow-growing chicken. This process may indicate that low-FE chicken tends to adapt to maintaining their blood glucose levels and simultaneously preserving their energetic status for metabolic purposes.

3.4.8 Proteins related to mitochondrial oxidative metabolism and oxidative stress

The majority of the energy production of a eukaryotic cell is generated through oxidative phosphorylation in the mitochondrial inner membrane (Bottje et al., 2006). The final phase of oxidative phosphorylation is carried out by ATP synthase or Complex V, one of the components of the electron transport chain. Interestingly, we observed three highly abundant proteins in the low-FE group: ATP synthase-coupling factor 6 (ATP5J), ATP synthase subunit d (ATP5H), and ATP synthase F1 subunit delta (ATP5D). These 3 proteins play an important role in the proton channel of ATP synthase facilitating electron flow through the respiratory chain and providing

energy for ATP synthesis. The same proteins were previously reported to be abundant in the rumen epithelium of L-RFI steer (Kong et al., 2016b) and in the breast muscle of L-RFI chickens (Yang et al., 2020). Thus, increased levels of these proteins suggest that the low-FE group may have increased energy production in the form of ATP in its duodenum, requiring a large quantity of energy. In addition, mitochondria are well recognized as a major source of reactive oxygen species (ROS), such as superoxide ($O_2^{\bullet-}$), produced by the electron transport chain during the process of oxidative phosphorylation. Some DAPs in our study are related to cell defense against ROS. For example, superoxide dismutase 1 (SOD1), which converts $O_2^{\bullet-}$ into hydrogen peroxide (H_2O_2) (Schäff et al., 2012), was more abundant in the low-FE group compared to the high-FE group. The over-production of SOD1 may result in elevated generation of H_2O_2 as observed in mitochondrial duodenal tissue (Ojano-Dirain et al., 2004) and in the breast (Bottje et al., 2006) of low-FE broilers. Furthermore, given that peroxiredoxin-1 (PRDX1) is important in cellular oxidative stress defense (Jeong et al., 2018), our findings (a lower level of PRDX1 in the low-FE group compared to the high-FE group) suggest that low-FE chickens are unable to remove excessive ROS as effectively as high-FE chickens and thus more likely suffer from oxidative damage than high-FE chickens. Peroxisomal metabolism, which is closely related to mitochondrial metabolism and immune response activation, is reportedly important for improving FE in poultry (Di Cara et al., 2019; Xiao et al., 2021). We thus hypothesized that proteins associated with the peroxisome pathway are responsible for modulating redox imbalance between ROS production and elimination caused by mitochondrial dysfunction, which contributes to oxidative stress in the chicken duodenum. Also, both mitochondrial inefficiency and oxidative stress may contribute to FE variation (Bottje and Carstens, 2009). In agreement with this, duodenal genes related to ROS production were over-represented in inefficient chickens (Yi et al., 2015) and beef cattle (Yang et al., 2021). Moreover, the failure of ROS detoxification can lead to intestinal inflammation and poor absorption (Mishra and Jha, 2019). Therefore, we suggest that greater susceptibility to oxidative stress may be responsible for the poorer FE in the low-FE group compared to the high-FE group.

3.4.9 Proteins related to intestinal nutrient permeability

The tight junction pathway is related to the physiological function of epithelial cells affecting the absorption of nutrients in the small intestine (Choct, 2009). The stability of tight junctions has an impact on the capacity of intestinal barrier permeability. Previous research showed that tight junction integrity and paracellular permeability were associated with the regulation of actin cytoskeleton and intercellular adhesion strength (Bruewer et al., 2004). In our study, three DAPs (ACTN1, SLC9A3R1, TUBA3E) were related to the tight junction pathway. Alpha actinins (ACTN1) and the tubulin alpha-3E chain (TUBA3E) were highly abundant in the high-FE group. This agrees with a transcriptome study showing upregulation of the genes encoding these proteins in the L-RFI epithelium of beef cattle (Kong et al., 2016b). The overproduction of these proteins suggest that high-FE chickens have greater intestinal integrity and epithelial function than low-FE chickens, possibly also resulting in greater paracellular nutrient permeability.

Tight junctions not only play a key role in nutrient absorption but also form a physical barrier against the external environments of the intestinal epithelial cell to prevent the entry of unwanted organisms, antigens, and toxins (Groschwitz and Hogan, 2009). Damage to the intestinal epithelial barrier can lead to inflammation (De Meyer et al., 2019). Thus, it is important for efficient production and optimal health (De Oliveira et al., 2018). Hypothetically, a better adaptive immune response requires less energy that can be used for growth (Horodyska et al., 2018).

Solute carrier family 9, subfamily A (SLC9A3R1) was highly abundant in the low-FE group. SLC9A3R1 is involved in several signaling pathways, such as cAMP-mediated phosphorylation that induces phosphorylation of claudin, a tight junction protein that is the major determinant of the barrier function (Chiba et al., 2008). Related to this, cAMP elevates the barrier function via PKA-dependent and independent pathways enhancing the junctional immunoreactivity of claudin and changing the barrier function of tight junction proteins (Chiba et al., 2008). Therefore, it is possible that the overproduction of the SLC9A3R1 protein may be related to the physiological adaptation of tight junctions to prevent the failure of its intestinal epithelium barrier function when gut health is compromised.

Based on our proteomics result, changes in metabolic activity, energy homeostasis, oxidative stress, and tight junction appear to play important roles in regulating FE. Previous studies showed that animals with poor FE require more energy for maintaining tissue homeostasis and have less usable energy for growth (Fonseca et al., 2019; De Lima et al., 2020). This may explain why the low-FE group gained less weight when consuming the same amount of feed as the high-FE group. Selecting for better FCR therefore promotes better feed conversion efficiency, growth, and production. In our study, we did not identify pathways involved in nutrient absorption. Dokladny et al. (2016) reported that microvilli are a crucial factor affecting the nutrient absorption capacity of the small intestine. Although our study detected some proteins related to the microvilli, such as plastin-1 (PLS1), vinculin (VCL), F-actin-capping protein subunit beta (CAPZB), F-actin-capping protein subunit alpha (CAPZA2), and actin-related protein 3 (ACTR3), the abundances of these proteins were either relatively low or did not differ between the two groups. Further studies focused on microvilli are required to profoundly understand the mechanism related to nutrient absorption.

3.5 Conclusions

Our results indicate that the different FE potential of slow-growing chicken is related to duodenal metabolism through proteins enriched in five main metabolic pathways: glycolysis/gluconeogenesis, peroxisome, oxidative phosphorylation, tight junction, and cysteine and methionine metabolism. These findings suggest that high-FE chickens have better glucose breakdown to extract energy for cellular metabolism from glycolysis and better tight junction strength of their intestinal epithelium than low-FE chickens. On the other hand, low-FE chickens may need to activate their amino acid metabolism and oxidative phosphorylation to provide more compensatory precursors for gluconeogenesis, to prevent disruption in their intestinal barrier function. These findings provide potential dietary energy-related biomarkers for selection to improve FE in chicken. However, given the relatively small number of biological replicates used in this study, further work is needed to confirm these findings.

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CHAPTER IV
THIGH MUSCLE METABOLIC RESPONSE IS LINKED TO FEED EFFICIENCY AND MEAT CHARACTERISTICS IN SLOW-GROWING CHICKEN

4.1 Abstract

The Korat chicken (KR) is a slow-growing Thai chicken breed with relatively poor feed efficiency (FE) but very tasty meat with high protein and low fat contents, and a unique texture. To enhance the competitiveness of KR, its FE should be improved. However, selecting for FE has an unknown effect on meat characteristics. Thus, understanding the genetic basis underlying FE traits and meat characteristics is needed. In this study, 75 male KR birds were raised up to ten weeks of age. For each bird, the feed conversion ratio (FCR), residual feed intake (RFI), and physicochemical properties, flavor precursors, and biological compounds in the thigh meat were evaluated. At ten weeks of age, thigh muscle samples from six birds (three with high FCR and three with low FCR values) were selected, and their proteomes were investigated using a label-free proteomic method. Weighted gene co-expression network analysis (WGCNA) was used to screen the key protein modules and pathways. The WGCNA results revealed that FE and meat characteristics significantly correlated with the same protein module. However, the correlation was unfavorable; improving FE may result in a decrease in meat quality through the alteration in biological processes including glycolysis/gluconeogenesis, metabolic pathway, carbon metabolism, biosynthesis of amino acids, pyruvate metabolism, and protein processing in the endoplasmic reticulum. The hub proteins of the significant module (TNNT1, TNNT3, TNNI2, TNNC2, MYLPF, MYH10, GADPH, PGK1, LDHA, and GPI) were also identified to be associated with energy metabolism, and muscle growth and development. Given that the same proteins and pathways are present in FE and meat characteristics but in opposite directions, selection practices for KR should

simultaneously consider both trait groups to maintain the high meat quality of KR while improving FE.

Keywords: Feed efficiency, Meat characteristics, Hub proteins, WGCNA, Slow-growing chicken

4.2 Introduction

The Korat chicken (KR) is a crossbreed between Thai indigenous line males (Leung Hang Khao: LHK) and synthetic Suranaree University of Technology (SUT) line females (Poompramun et al., 2021b). KR is known for its healthy meat, with rich flavor and unique texture that are valued by consumers (Katemala et al., 2021). However, poor feed efficiency (FE) of KR increases production costs and reduces the competitiveness of KR against other commercial chicken commonly used in Thailand. To improve profitability and competitiveness, a balance between FE and meat characteristics is an important breeding goal for KR.

Traditionally, FE is measured as a feed conversion ratio (FCR); a ratio of feed intake (FI) to body weight gain (BWG). Selecting for FCR more efficiently improves the numerator trait (FI) compared with the denominator trait (BWG), a well-known limitation of FCR that causes nonlinear selection pressure (Gunsett, 1984). Residual feed intake (RFI) is another commonly used FE measurement; it is the difference between observed and expected feed intake based on requirements for maintenance and growth (Koch et al., 1963). Due to its low genetic correlation with production traits and a moderate correlation with FCR and FI, RFI is considered the most appropriate trait for the genetic improvement of energy efficiency in poultry (Willems et al., 2013; Xu et al., 2016). However, improving FE potentially causes a decrease in meat quality and reduces consumer acceptance of meat (Zhou et al., 2015). In addition, FE is age dependent in chicken (Arthur et al., 2001; Berry and Pryce, 2014) due to developmental processes and maturity of the physiological functions of the tissues (Aggrey et al., 2010; Ravindran and Abdollahi, 2021).

Previous skeletal muscle transcriptomic analysis in chicken have indicated that FE may be related to nucleotide sugar biosynthesis, glycogen metabolism, and lipid uptake and transport (Abasht et al., 2019). Our previous transcriptomic analysis with

KR chicken indicated that nucleotide metabolism, fatty acid metabolic process, and oxidative stress play key roles in regulating both FE and the quality of thigh meat (Poompramun et al., 2021a). Thus, improving FE can have a negative impact on meat texture and the nutritional value of meat via activating the accumulation of biochemical compounds and flavor precursors. Although the molecular mechanisms have been extensively investigated at the transcriptome level (Poompramun et al., 2021a), understanding is lacking on how the FE and thigh muscle meat quality of this slow-growing chicken breed are linked at the proteomics level. Overall, a better understanding of how FE affects meat characteristics is crucial to avoiding any unfavorable effects of improving FE on the quality and characteristics of KR thigh meat.

In this study, we use label-free proteomics to profile the thigh muscle proteome of KR with either high or low FE and to identify key proteins and molecular pathways related to both FE and meat characteristics. Our main hypotheses are: 1) the regulation of FCR and RFI depend on several molecular and physiological mechanisms, 2) a negative relationship exists between FE and meat characteristics, and 3) the molecular and physiological determinants of FCR and RFI are age dependent. New information gained can be used in selection strategies to improve FE while retaining the excellent meat characteristics of KR.

4.3 Materials and methods

4.3.1 Ethics statement

The experiment was carried out at Suranaree University of Technology's experimental farm. All experimental procedures were approved by The Ethics Committee on Animal Use constituted by the Suranaree University of Technology in Nakhon Ratchasima, Thailand (U1-02631-2559).

4.3.2 Experimental animals and tissue collection

Seventy-five male KR were used in this experiment. The birds were produced by mating individuals with the highest body weights together and by mating individuals with the lowest body weights together. At hatching, the birds were sexed using the vent sexing method, individually weighted, wing banded, and vaccinated against Marek's disease. Subsequently, they were vaccinated following the recommendation of the Department of Livestock Development, Thailand. All birds

were individually raised in the same conditions in 63 × 125 × 63 cm cages covered with rice hulls. The diet of birds 0 to 3 weeks of age included 21% protein (a starter diet), 19% protein for birds 4 to 6 weeks of age (a grower diet), and 17% protein for birds 7 to 10 weeks of age (a finisher diet). Birds were fed ad libitum and water was freely available in each cage through nipple drinkers.

At 10 weeks of age, all birds were transported to the slaughterhouse, rested for 30 minutes, and electrocuted before having their necks cut, bled, and plucked. The carcasses were manually eviscerated and washed before being stored in a cold room (4°C). A piece of breast and thigh muscle of each bird was collected, snap frozen in liquid nitrogen, and stored at −80°C until further proteome analyses.

4.3.3 Feed efficiency

Feed efficiency was determined using two measurements: FCR and RFI. The FCR was calculated for each bird i and week k as $FCR_{ik} = FI_{ik}/BWG_{ik}$, where FI_{ik} is the total feed intake of bird i from hatch to week k and BWG_{ik} is the BWG of the same bird for the same period. Only FCRs for weeks 2, 4, 6, 8, and 10 were used in the statistical analysis.

The RFI were first calculated for different weeks using the following formula:

$$RES_{ij} = FI_{ij} - (b_0 + b_1 MMW_{ij} + b_2 BWG_{ij})$$

where FI_{ij} is the total feed intake of bird i during week j, MMW_{ij} is the metabolic weight of bird i calculated from mean body weights at weeks j and j-1 $\left(\frac{BW_{ij}+BW_{ij-1}}{2}^{0.75}\right)$, BWG_{ij} is the body weight gain between the weeks j and j-1, b_0 is the intercept, and b_1 and b_2 are partial regression coefficients. For the statistical analysis the weekly RFI's were combined i.e. for bird i, RFI for week k is $RFI_{ik} = \sum_{j=1}^{k} RES_{ij}$. Again, in the statistical analysis only RFI of weeks 2, 4, 6, 8, and 10 were used.

In the present study, six male KR exhibiting the most extreme FCRs at 10 weeks of age (FCR_{10}) were chosen for further proteomic analyses: three with the highest FCRs (FCR_{10} = 3.33, 3.34, and 3.36) and three with the lowest FCRs (FCR_{10} = 1.83, 1.98, and 1.99). These groups are later referred to as the low-FE and high-FE

groups, respectively. The studied birds were the same as in our previous gene expression study of the KR thigh muscle (Poompramun et al., 2021a).

4.3.4 Meat characteristics measurements and chemical analyses

For meat quality measurement, breast and thigh meat samples were collected 24 h after chilling. We consider only those meat characteristics that relate to the texture and flavor of the meat: ultimate pH (pHu), water holding capacity (WHC), and drip loss (DL). In addition, chemical analysis was conducted for adenosine triphosphate (ATP)-related traits (guanosine monophosphate (GMP), inosine monophosphate (IMP), adenosine monophosphate (AMP), and inosine (Ino). Moreover, the biomolecule profiles of lipids, amide I, amide II, amide III, CH-binding of lipid, carbohydrate, and glycogen obtained from the Fourier Transform Infrared (FTIR) were used to reveal any changes in key biomolecules related to FE. For more details concerning sample collection, preparation, and measurements, see the previous study by Poompramun et al. (2021a).

4.3.5 Protein analysis

Breast and thigh muscle tissue from six samples were freeze-dried and grounded into a fine powder. Samples were lysed in denaturing buffer (50 mM ammonium bicarbonate containing 8M urea: AMBIC, Sigma-Aldrich, St. Louis, MO) using the sonication method. The lysates were clarified by centrifugation at 20,000 g for 10 min at 4˚C. The collected proteins were diluted with 50 mM AMBIC (final concentration of 1.5 M urea). Protein quantifications were then determined with the Pierce BCA Protein Assay kit (Thermo Fisher Scientific, Waltham, MA). Proteins were reduced with dithiothreitol (a final concentration of 5 mM) for 20 min at 50-60˚C. Then, iodoacetamide (final concentration of 15 mM) was added to the protein samples and incubated for 20 min at room temperature in the dark environment, followed by protein digestion with 2 µg of trypsin at 37˚C for 24 h.

Peptide mixtures were analyzed using a Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA) at the Proteomics Unit core facility, University of Helsinki, Finland. The peptides were loaded onto a C18 reverse-phase column on an 80-min gradient. MS data were operated using a data-dependent acquisition (DDA) mode and higher-energy

collisional dissociation (HCD) for mass fragmentation. One technical replicate from each six samples was combined and analyzed for the database search.

Raw MS files from Orbitrap mass spectrometry were processed using version 1.6.5.0 of MaxQuant (Cox and Mann, 2008). The mass spectra were annotated against the Uniprot Gallus gallus database (34 925 total entries, downloaded from https://www.uniprot.org, January 2019 version). Two missed cleavages were allowed for trypsin specificity. Data searches were conducted with variable modifications of oxidation (M) and acetyl (protein N-term), and carbamidomethylation was set as a fixed modification. The initial mass precursor tolerance was set to 20 ppm and 6 ppm in the first search and main search, respectively. Furthermore, fragment (MS/MS) mass deviation was set to 20 ppm. The false discovery rates (FDR) for peptide and protein identification were set to 0.01. Protein abundance was defined by the normalized spectral protein intensity (LFQ intensity).

4.3.6 Differential proteomic analysis

Label-free quantification intensity values were transformed to a logarithmic scale with a base of 2. Missing values were imputed from a normal distribution (width: 0.3, down shift: 1.8). The DEqMS R package version 1.11.1 was used to analyze the differential abundance proteins (DAPs) in the muscle tissue between high- and low-FE chickens (Zhu et al., 2020). Proteins with a P-value <0.05 were considered DAPs. Hierarchical clustering of DAPs was generated using the pheatmap R package version 1.0.12. The visualization of the differences and similarities of the proteomic profiles and DAPs between the high- and low-FE groups was constructed using a principal component analysis.

4.3.7 Protein network construction and module identification

Bioinformatic analyses, i.e., protein networks construction, highly correlated proteins cluster (module) identification, and their further analysis were based on Wight Gene Co-expression Network Analysis (WGCNA), available in the R package (Langfelder and Horvath, 2008).

To specify an unsigned network, a weighted adjacency matrix was formed based on absolute values of the Pairwise Pearson's correlation coefficients among all proteins. The soft threshold power (β) was set to 9 to reach the scale-free topology criterion (R2 = 0.80). The topological overlap measure (TOM) and the

corresponding dissimilarity (1−TOM) were calculated using the adjacency matrix. The modules of co-expressed proteins were identified by the Dynamic Tree Cut algorithm. The minimum size of the module was set to 30 proteins. Modules that were very similar were merged (height cut-off of 0.15 in the dendrogram). Finally, each module was labeled with different colors, and proteins that did not belong to any of the modules were grouped together to form their own module (grey).

4.3.8 Module-Trait Relationship

The module eigenproteins (ME), defined as the first principal components of the modules (Langfelder and Horvath, 2008), were used to study the relationships between the modules and FE and meat characteristics including chemical compounds. The relationships were based on Pearson's correlations and only modules that were significantly ($P < 0.05$) correlated with FE and meat characteristics were used in the functional enrichment analysis.

4.3.9 Functional enrichment analysis of proteins

To gain insight into the potential biological function of the identified proteins, all proteins from the selected module were subject to the gene ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis using STRING (version 11.5, http://string-db.org, Szklarczyk et al., 2015). Functional enrichment was carried out in three GO categories: biological process (BP), molecular function (MF), and cellular component (CC). The GO results were visualized using the R package "ggplot2" (version 3.3.5, https://ggplot2.tidyverse.org/). Enriched GO proteins and KEGG pathways with FDR-adjusted (Benjamini-Hochberg method) *P*-values < 0.05 were considered statistically significant.

4.3.10 Hub protein identification

Hub proteins were characterized as proteins with the highest connectivity. Hub proteins were identified based on their protein significance (PS ≥ 0.6), corresponding to the absolute value of the correlation between the protein expression profile and the traits of interest, and module membership (MM ≥ 0.7), defined as the correlation between the MEs and its protein abundance profile. Subsequently, the hub proteins were provided as input into Cytohubba, a Cytoscape plugin (version 3.6.1, https://cytoscape.org/), to identify the highest linkage hub

proteins in the network based on the Maximal Clique Centrality (MCC) algorithm (Chen et al., 2009).

4.4 Results

4.4.1 Proteomic differences between FE groups

Breast and thigh tissues from six samples of KR with different FE (n=3 per FCR group) were collected for proteomic analysis. After filtering, 303 and 313 proteins were identified in breast and thigh tissue, respectively, and used for differential abundance protein analysis. Nevertheless, no significantly differential abundance proteins (DAPs) were detected in the breast tissue between the two different groups. It might be due to the high variation within each group, as shown in figure 4.1. Principal component analysis (PCA) of the breast proteome data revealed that the clusters of the high-FE group and the low-FE group largely overlapped, indicating there was not clearly discrimination between these two groups. While the over 39 % of the proteome variation were observed in the thigh tissue between two difference group, revealed significant differences between high- and low-FE groups, so we decided to compare the differences of the thigh proteome abundance level between two groups of chicken.

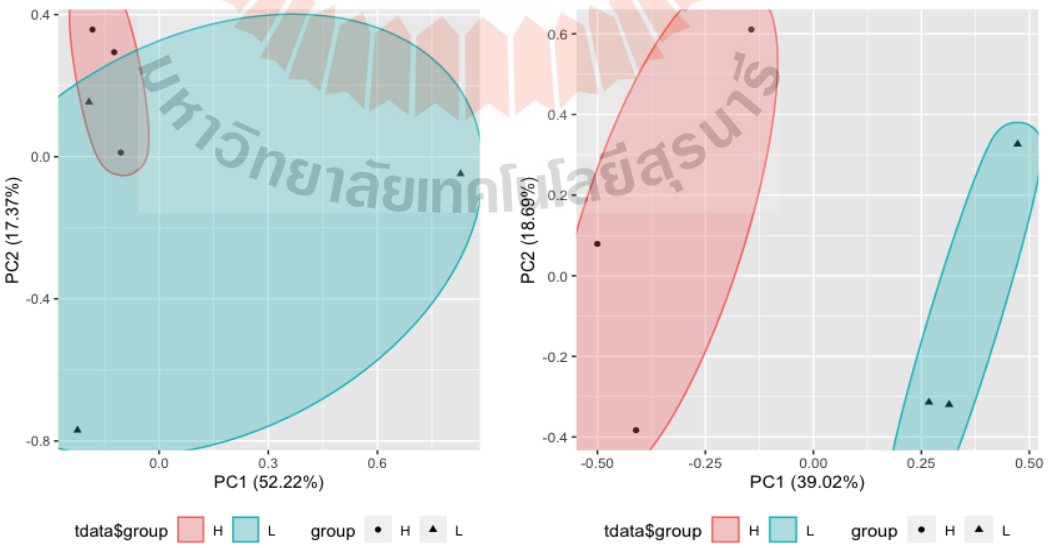

Figure 4.1 Principal component analysis (PCA) of the breast protein samples (left) and the thigh protein samples (right) from high-and low-FE chicken.

In the thigh, a total of 75 proteins were identified as being DAPs (P-value < 0.05). Of the 75 proteins, 49 DAPs had high abundance in the high-FE groups, while 26 had low abundance in the low-FE groups (Figure 4.2). A heatmap of the DAPs indicated that these DAPs easily differentiated the high-FE group from the low-FE group. Thus, we will use the proteomic data of the thigh muscle for further analysis.

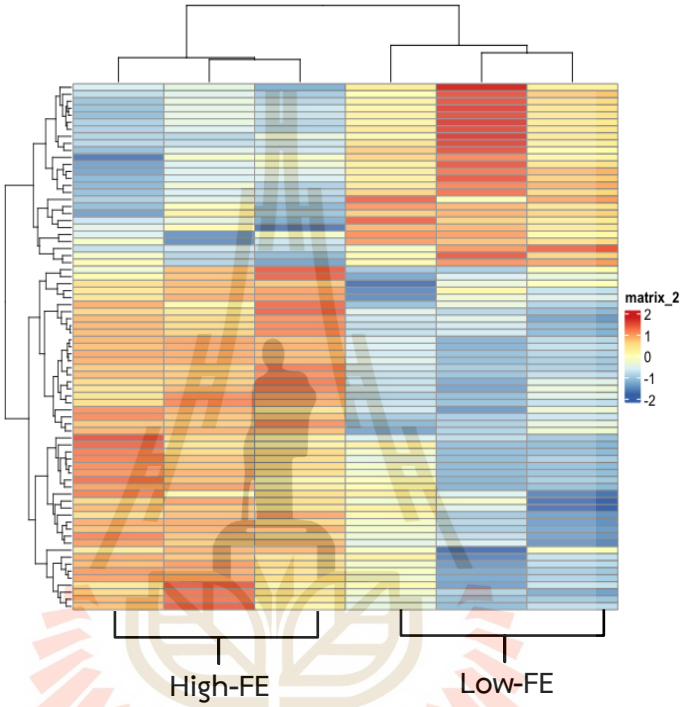

Figure 4.2 The heat map of the 75 DAPs between the high- and the low-FE group. Abbreviation: DAPs, differentially abundant proteins.

4.4.2 Co-expressed protein network construction and key module identification in the thigh muscle

A total of 904 proteins were detected from six thigh muscle tissue samples of KR. Their abundance profiles are presented in the Supplementary Table 4.1. After filtering, 313 proteins remained and were used to explore the association between the proteomic profiles and the FE and meat characteristic traits using WGCNA. Four modules were identified (blue, brown, turquoise, and yellow) with 53, 42, 127, 35 co-expressed proteins, respectively. The "grey" module included proteins that were not clustered.

The association between protein modules and FE and meat characteristic traits is illustrated in Figure 4.3. The turquoise module had the highest number of significant associations with FE and meat characteristics (Figure 4.3). A negative correlation was found between the turquoise module and FCR at 4, 6, 8, and 10 weeks of age and between the turquoise module and RFI at 6, 8, and 10 weeks of age. Thus, the higher the quantity of the eigenproteins in the turquoise module, the better FCR and RFI values the birds had. In addition, the turquoise module had a negative correlation with WHC and a positive correlation with inosine, amide I, and C-H bending. As the turquoise module showed the strongest association with FE and meat characteristics traits, we focus only on the turquoise module in the subsequent analyses.

4.4.3 Functional enrichment analysis of the turquoise module

Based on the GO analysis, proteins in the turquoise module were mainly localized in the intracellular (GO:0005622), cytoplasm (GO:0005737), intracellular non-membrane-bounded organelle (GO:0043232), cytosol (GO:0005829), and cytoskeleton (GO:0005856) (Figure 4.4). The molecular functions were mainly involved in cytoskeletal protein binding (GO:0008092), actin binding (GO:0003779), protein-containing complex binding (GO:0044877), structural molecule activity (GO:0005198), and lyase activity (GO:0016829) (Figure 4.5). The enriched biological processes were small molecule metabolic process (GO:0044281), cytoskeleton organization (GO:0007010), organophosphate metabolic process (GO:0019637), actin cytoskeleton organization (GO:0030036), and carbohydrate metabolic process (GO:0005975) (Figure 4.6), which mostly relate to metabolic processes within the muscular cell.

Based on the KEGG analyses, the turquoise module was highly enriched with proteins related to substance or energy metabolism, including the “glycolysis/gluconeogenesis”, “biosynthesis of amino acids”, “pyruvate metabolism”, “metabolic pathway”, and “carbon metabolism” pathways (Table 4.1). Our results show that the processes related to energy generation in the thigh muscles of slow-growing chicken are related to FE, muscle metabolism, biomolecules, and flavor precursors and meat characteristics.

4.4.4 Candidate hub proteins screening

The turquoise module was comprised of 127 proteins (Supplementary Table 4.2). Among them, proteins that had the strongest correlation (|PS| ≥ 0.6) with FE and meat characteristics traits and the module eigenproteins (|MM| ≥ 0.7) were identified as candidate hub proteins (Supplementary Table 4.3). Pathway enrichment was further conducted to understand the biological role of these identified hub proteins. Cytohubba revealed 10 hub proteins that were highly connected: TNNT1 (slow skeletal muscle TnT), TNNT3 (fast skeletal muscle TnT), TNNI2 (Troponin I2), TNNC2 (Troponin C), MYLPF (myosin light chain, fast skeletal muscle), MYH10 (Myosin Heavy Chain 10), GADPH (Glyceraldehyde-3-phosphate dehydrogenase), PGK1 (Phosphoglycerate kinase), LDHA (L-lactate dehydrogenase A chain), and GPI (Glucose-6-phosphate isomerase) (Figure 4.7). These proteins were considered key regulators for both FE and meat characteristics.

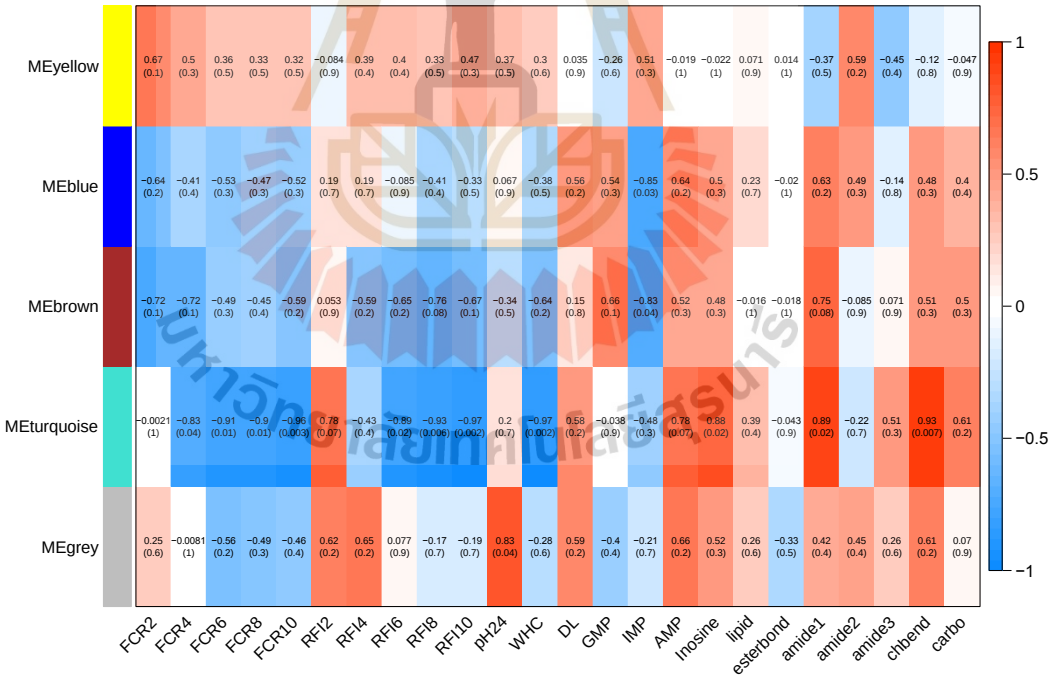

Figure 4.3 Correlations between protein modules and feed efficiency and meat characteristics traits. The module names are given in the y-axis, and the traits in the x-axis. The table is color-coded by correlation values: blue color represents a negative correlation, while red color represents a positive correlation. The numbers in each cell are correlation coefficients

and the P-values (in parentheses). FCR 2, 4, 6, 8, and10; feed conversion ratio at 2, 4, 6, 8, 10 weeks of age; RFI 2 , 4, 6, 8, and10, residual feed intake at 2,4,6,8,10 weeks of age; WHC, water-holding capacity; DL, drip loss, GMP, guanosine monophosphate; IMP, inosine monophosphate; AMP, adenosine monophosphate; Esterlipid, ester carbonyl of phospholipids; chbend, CH-bending; and Carbo, carbohydrate and glycogen.

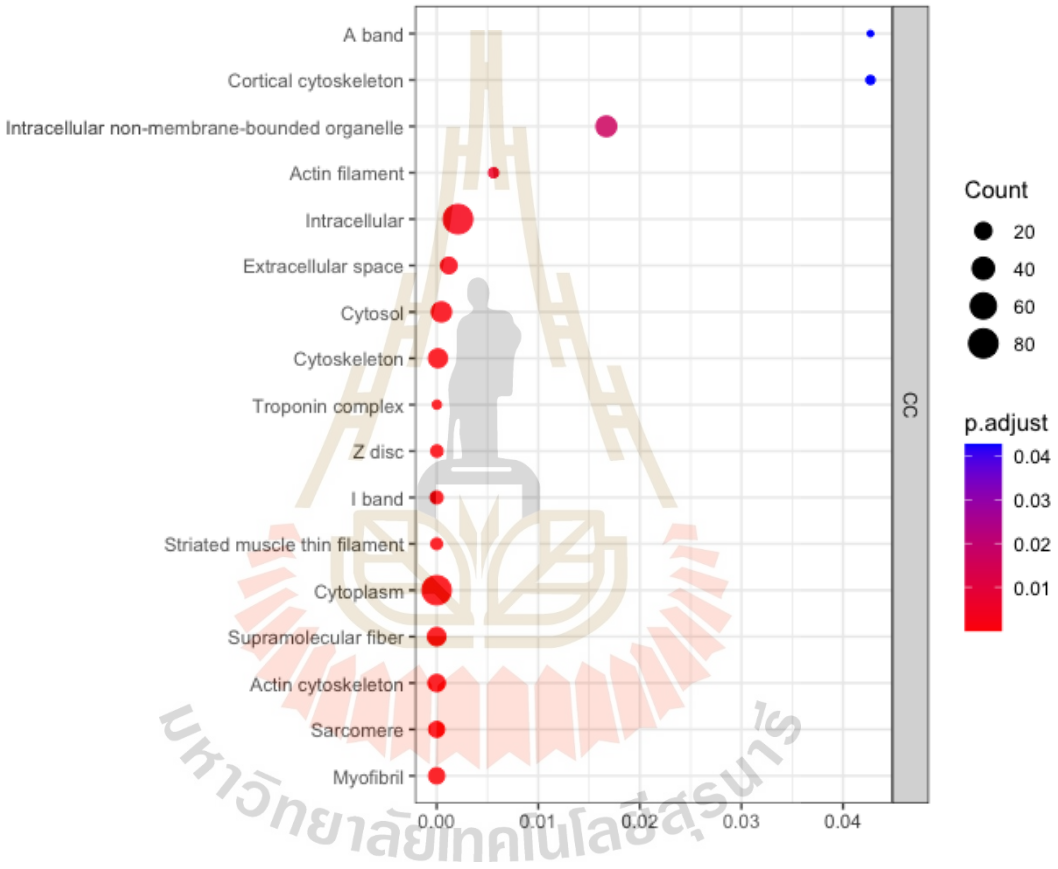

Figure 4.4 Gene Ontology enrichment analysis of cellular components for proteins in the turquoise modules.

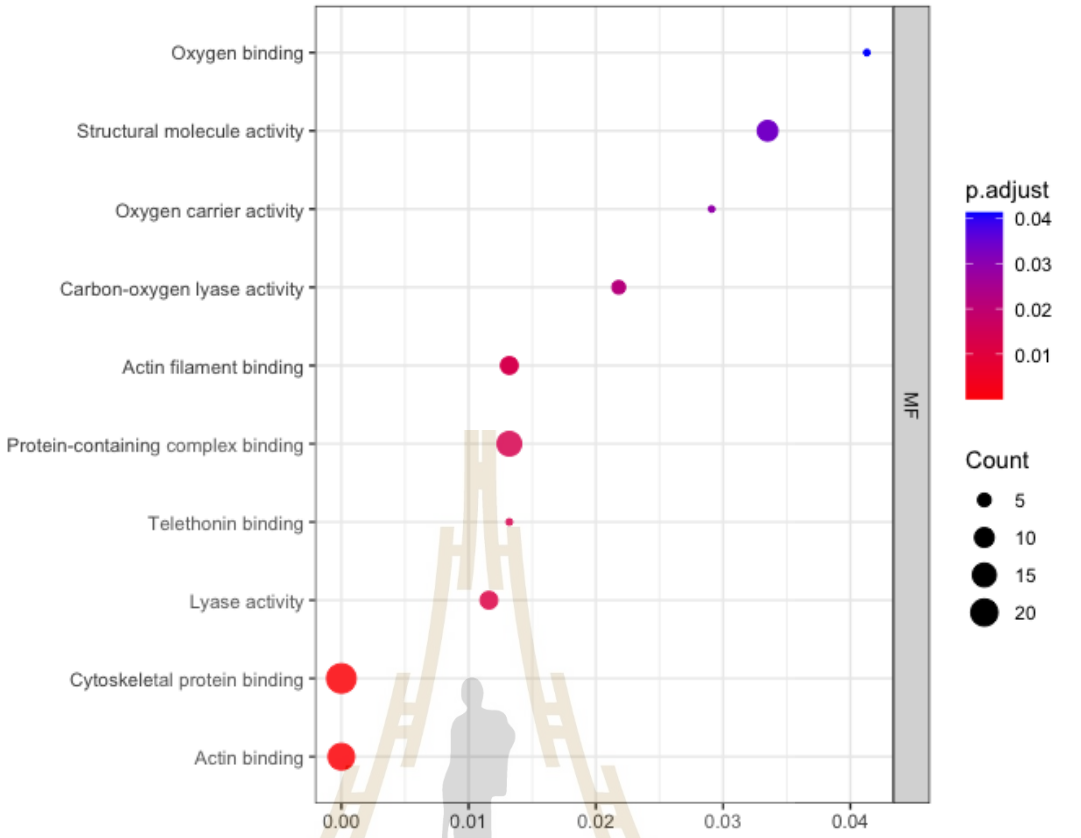

Figure 4.5 Gene Ontology enrichment analysis of molecular function for proteins in the turquoise modules.

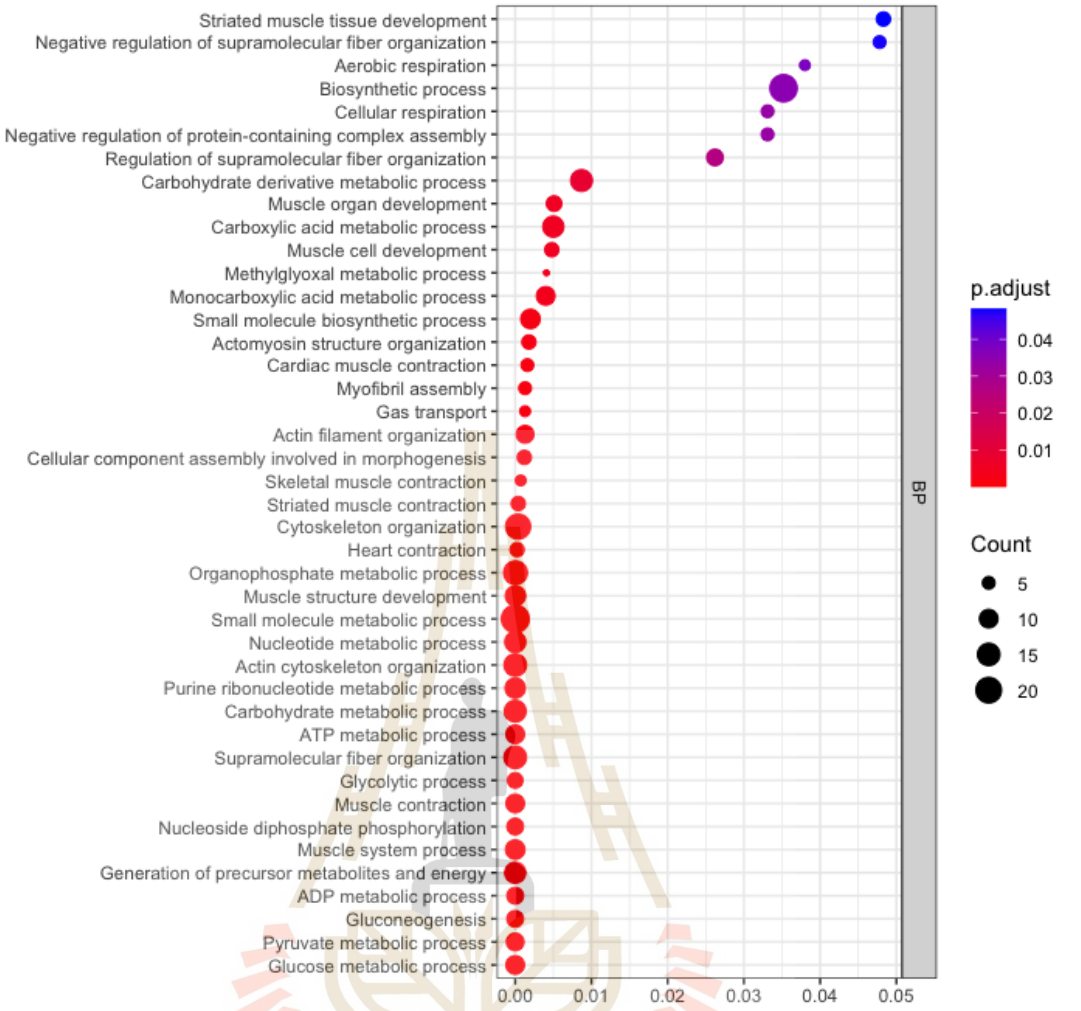

Figure 4.6 Gene Ontology enrichment analysis of biological processes for proteins in the turquoise modules.

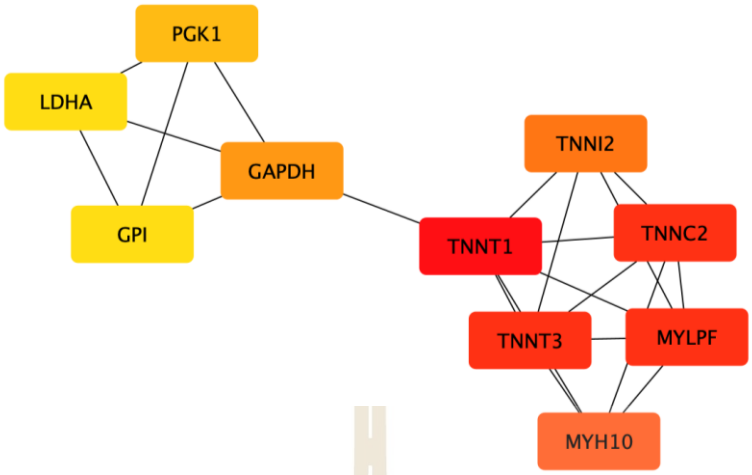

Figure 4.7 The connectivity plot of the 10 hub proteins using CytoHubba plugin. The color of a node indicates the degree of connectivity (red for high degree, orange for intermediate degree, and yellow for low degree).

Table 4.1 A summary of the KEGG pathways for proteins in the turquoise module.

KEGG ID	Description	Protein count[1]	p- adjust[2]
00010	Glycolysis / Gluconeogenesis	8	3.67E-10
01200	Carbon metabolism	8	3.40E-08
01230	Biosynthesis of amino acids	7	3.40E-08
00620	Pyruvate metabolism	4	8.37E-05
01100	Metabolic pathways	14	8.37E-05
04141	Protein processing in endoplasmic reticulum	4	0.0159

[1]The number of proteins associated with KEGG terms.

[2]FDR-adjusted P-values.

4.5 Discussion

4.5.1 Differential protein abundance

Feed efficiency is a complex trait that is influenced by feed- and growth-related factors. Thus, to study the mechanism underlying FE in the skeletal muscle of slow-growing chickens, we identified the proteome differences between high-FE and low-FE chickens using the label-free proteomic method. Unexpectedly, we found that proteome abundance between the two groups of chickens exhibited

no significant difference in the breast muscle, whereas a total of 75 DAPs were observed in the thigh muscle between the two groups. Skeletal muscle fibers are generally divided into oxidative (slow-twitch, type I) and glycolytic fibers (fast twitch, type IIA, IIB) according to the myosin heavy chain (MyHC) isoforms in chickens (Ono et al., 1993). It has been reported that the breast muscle mainly consists of 100 % glycolytic fibers, whereas leg muscle contains both glycolytic and a few oxidative fibers (Weng et al., 2022). The muscle's metabolic properties are normally determined by the muscle fibers characteristics, like myofiber area and myofiber density, and fiber type composition (Ismail and Joo, 2017). Previous study showed that there was no significant difference in myofiber area and density in breast muscle between the fast-growing line and the slow-growing line. While the myofiber area was significantly increased and myofiber was lower in the thigh muscle in fast-growing line than slow-growing line (Shao et al., 2022), indicating that growth has greater influence on thigh muscle than breast muscle. Moreover, it has been reported that the development of myofiber types is influenced by functional demands on skeletal muscle associated with increasing body weight (Aberle and Stewart, 1983). Thus, the reason that we did not observe any impact of FE on proteomic profile in the breast muscle in the current study, most likely because breast and thigh muscles have different metabolic properties. However, more research is needed to investigate the differences of muscle morphology between breast and thigh muscle in the Korat chicken.

In addition, improving FE and meat characteristics are crucial for the profitable and competitive chicken production. Leg muscles (thigh and drumstick) are more popular in East Asia, Mexico, India, Russia, and Morocco (Willems, 2018). However, the relationship between FE and meat characteristics in the thigh muscle of slow-growing chicken and the mechanism behind it is still largely unknown. Therefore, the aim of this study was to identify the role of the key regulator proteins and pathways underlying FCR and RFI in different development stages and meat characteristics such as physiological properties, flavor indicators, and biomolecules.

4.5.2 Key pathways identification

We presented three main hypotheses: 1) the regulation of FCR and RFI depend on several molecular and physiological mechanisms, 2) a negative relationship exists between FE and meat characteristics, and 3) the molecular and

physiological determinants of FCR and RFI are age dependent. Surprisingly, our results did not support the first hypothesis, only one module (turquoise) was associated with FCR and RFI. Pathways related to proteins of the significant module, including glycolysis and gluconeogenesis, metabolic pathway, carbon metabolism, amino acid biosynthesis, pyruvate metabolism, and protein processing in the endoplasmic reticulum (ER), are part of the complex biological events of protein biosynthesis, energy generation, and energy storage in the skeletal muscle.

Glycolysis/gluconeogenesis was previously reported to be one of the significant pathways associated with FE (Fonseca et al., 2019; Kaewsatuan et al., 2022). Glycolysis is the cytoplasmic pathway that efficiently produces ATP (energy) from glucose by converting it into two pyruvate molecules. Pyruvate can be completely oxidized in the mitochondria to generate ATP via the TCA cycle and oxidative phosphorylation, while gluconeogenesis is the metabolic process through which glucose is synthesized from non-carbohydrate precursors. These pathways play a key role in regulating the glucose and energy homeostasis of tissues with high metabolic demand, such as the skeletal muscle (Guo et al., 2012).

Moreover, the biosynthesis of amino acids and protein processing in the ER pathway were observed to have a significant effect on the FE of animals. (Fonseca et al., 2019; Pezeshkian et al., 2022). Amino acids are fundamental components of body proteins. Once amino acids enter a cell, they can be used as precursors for energy production or used for other biochemical processes, such as protein synthesis, depending on metabolic requirements (White et al., 2021). Protein synthesis is primarily regulated at the initiation phase of protein translation in the cytosol and ER compartment (Stephan et al., 2005). The ER is the main organelle responsible for calcium homeostasis, quality control, protein synthesis, and protein folding. The accumulation of unfolded or misfolded proteins causes stress to the ER (Marques et al., 2021). An increase in this pathway possibly increases the ER's capacity for facilitating protein folding and synthesis to restore protein homeostasis.

4.5.3 The association between key pathways and FE traits

Selecting for lower FCR increases body weight gain and leads to slightly higher feed intake (Yi et al., 2018). One possible explanation for the relationship between energy metabolism-related pathways, protein synthesis, and FCR may be

that a weight gain typically leads to changes in body composition, including a significant increase in metabolic tissues (e.g., muscle protein mass), which contributes to energy use in animals (Galgani and Ravussin, 2008). The increase in body weight is generally due to an increase in muscle fiber size (hypertrophy) and a greater number of muscle fibers (hyperplasia) (Wang et al., 2020b). The rate of muscle protein accumulation depends on the balance between energy intake and expenditure; the excess of substrate and energy intake lead to an increase in muscle protein deposition (Van Milgen et al., 2001). Meanwhile, selecting for RFI reportedly associated with differences in feed intake, feed digestion, metabolism, and thermoregulation (Herd and Arthur, 2009). As feed intake increases, a larger amount of energy is required to supply for the digestion processes in terms of increased digestive organ size and increased energy consumed for supporting tissue activities. Thus, an animal with a lower feed intake is expected to be more efficient because less energy is expended on tissue maintenance and activities (Herd and Arthur, 2009).

Our results showed that the turquoise module was negatively associated with FCR and RFI, indicating that greater efficiency in high-FE chicken may be due to an increased rate of glycolytic potential and protein-synthesizing potential. Efficient animals reportedly accumulate more muscle mass than inefficient animals (Xu et al., 2020). High-FE chicken are speculated to use more glucose to generate ATP for protein deposition and for maintaining tissue homeostasis.

4.5.4 The association between key pathways and meat characteristics

In addition to the negative correlation between the turquoise module and FE, the turquoise module showed a negative correlation with WHC and positive correlations with inosine, amide I, and C-H bending. Given that the same proteins and pathways are correlated with FE and meat characteristics but in opposite directions, changes in FE can negatively impact the meat characteristics, flavor, and biomolecules in thigh meat through alterations to metabolic processes, *i.e.,* glycolysis and gluconeogenesis, metabolic pathway, carbon metabolism, amino acid biosynthesis, pyruvate metabolism, and protein processing in the ER. These findings are in line with our second hypothesis.

After slaughter, the muscle temporarily converts pyruvate into lactate to maintain the homeostasis of ATP concentration when oxygen is limited. During the

post-mortem period, ATP can be degraded into adenosine diphosphate (ADP), AMP, IMP, and other derivative compounds. IMP gradually changed into inosine, causing a bitter taste in the meat (Ishiwatari et al., 2013; Dashdorj et al., 2015). Thus, the increased activity of the glycolysis/gluconeogenesis pathway may be a key factor in promoting inosine accumulation in the thigh meat of high-FE chicken and may cause unfavorable flavor in the meat.

Glycolysis and pyruvate metabolism elevations result in the accumulation of lactic acid, which consequently reduces the pH value of the post-mortem meat (Vanderhout et al., 2022). The higher the pH decline rate in meat, the greater the denaturation of proteins (Stajkovic et al., 2019). We speculated that the protein denaturing induces changes in the biochemical compounds of thigh meat, resulting in a higher amide I content and greater C-H bending in meat. According to Suwanvichanee et al. (2022) and Katemala et al. (2022), the amide I adsorption band is associated with changes in the secondary structure of the proteins (e.g., α-helical and β-sheet structure) and hydrophobic interactions of aliphatic amino acid residues, respectively. Our hypothesis is that an increase in amide I and C-H bending may associate with reduced hydrophobic core stability of the secondary structure of the proteins, resulting in increased denatured protein structure, decreased WHC, and consequently leading to an undesirable texture of the meat. Therefore, our findings indicate that the improvement of FE could potentially have an unfavorable effect on the texture and flavor of meat. Again, these results are in line with our second hypothesis

4.5.5 Key Hub proteins identification

Of the obtained hub proteins, TNNT1, TNNT3, TNNI2, TNNC2, MYLPF, MYH10, GADPH, PGK1, LDHA, and GPI showed high degrees of connectivity and can be considered key regulators of both FE and meat characteristics (Figure 4.7). TNNT1 (slow skeletal muscle TnT) and TNNT3 (fast skeletal muscle TnT) are the tropomyosin-binding subunits that comprise the elongated portion of the troponin complex (Tn-complex) (Marston and Zamora, 2020). TNNI2 (Troponin I2; TnI) is the inhibitory subunit of the Tn-complex, and TNNC2 (Troponin C) is the Ca2+-binding subunit of the Tn-complex. These three subunits compose the Tn-complex structure, which has an important role in the calcium-dependent regulation of skeletal muscle contraction and relaxation (Wei and Jin, 2016). Mutations in the three TnT isoform

genes (i.e., *TNNT1*, *TNNT2*, and *TNNT3*) have been found in cardiac and skeletal myopathies, suggesting that TnT plays a key role in striated muscle growth and function (Sheng and Jin, 2014).

MYLPF (myosin light chain, fast skeletal muscle) is reportedly associated with skeletal muscle tissue development (Wang et al., 2007). In zebrafish *(Danio rerio),* knockout of *mylpfa* causes degeneration of differentiated skeletal myofibers (Chong et al., 2020). The MYH10 protein (Myosin Heavy Chain 10) regulates cytokinesis, cell motility, and cell polarity (Ridge et al., 2017). PGK1 (Phosphoglycerate kinase) is known for the first ATP-yielding step via a PGK-catalyzed reaction, which participates in the reversible reaction from 1,3-bisphosphoglycerat to ADP to form 3-phosphoglycerate and ATP, catalyzed by phosphoglycerate kinase (Alto et al., 2021). Kierans and Taylor (2020) reported that the *PGK 1* gene was upregulated under hypoxia conditions, suggesting that an increase in glycolytic activity during hypoxia stress may help preserve bioenergetic balance. GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) is an anaerobic regulator of glycolysis and is widely used as an internal control when comparing gene and protein expression levels. It catalyzes the reversible conversion of glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate during glycolysis (Wang et al., 2020a). GPI (Glucose phosphate isomerase) catalyzes the reversible reaction of glucose-6-phosphate to fructose-6-phosphate (Haller et al., 2010). Knocking down *GPI* gene expression can decrease the glycolytic gene activity and endogenous glucose level in PGCs in the primordial germ cells (PGCs) of chicken (Rengaraj et al., 2012). Additionally, LDHA (Lactate Dehydrogenase A) catalyzes the reversible conversion of pyruvate to lactate and acts as an indicator of glycolytic capacity (Zhao et al., 2020). LDHA reportedly plays a key role in lactate homeostasis and energy balance and was previously identified as a candidate gene for muscle development (Qiu et al., 2010). Because of their functions, these discovered hub proteins may potentially affect FE and meat characteristics.

The turquoise module correlated significantly with FCR for weeks 4 to 10 and with RFI for weeks 6 to 10 (Figure 4.3 and Supplementary Table 4.3), indicating age dependency and supporting our third hypothesis. Consistently, Liu et al. (2016) reported that pathways involved in muscle development, including hypertrophic cardiomyopathy, cardiac muscle contraction, tight junctions, and focal adhesion, were

prominent between days 56 and 98 in Beijing-You chicken. However, it is necessary to examine the expression of these 10 hub proteins at various ages to deepen our understanding of the molecular mechanism underlying age-dependent FE.

4.6 Conclusion

To our knowledge, this is the first study that analyzes the complex protein regulation system underlying FE and meat characteristics in a slow-growing chicken breed. In this study, WGCNA was applied to detect the key proteins affecting both FE and meat characteristics using proteomic data of the thigh muscle. The ten most important hub proteins obtained (TNNT1, TNNT3, TNNI2, TNNC2, MYLPF, MYH10, GADPH, PGK1, LDHA, and GPI) play a critical role in molecular responses involved in energy metabolism and muscle development. In addition, our findings provide evidence that improving FE may have an unfavorable effect on the texture and flavor of thigh meat, which should be accounted for in the selection programs of meat-producing chicken breeds

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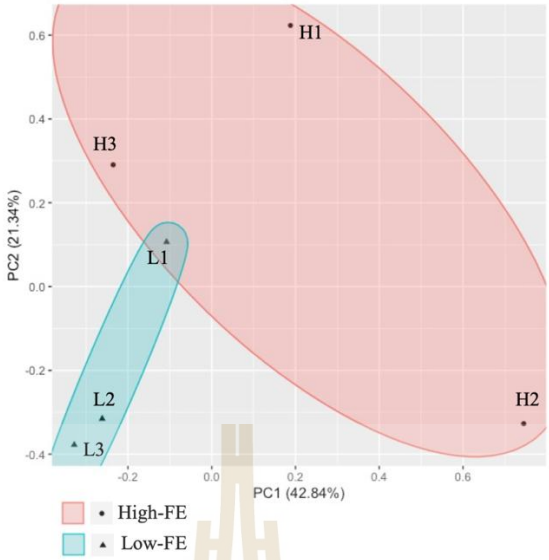
CHAPTER V
SUMMARY

Our study provides a new insight into molecular mechanisms controlling the regulation of feed efficiency in slow-growing chickens in both digestive tissue level such as duodenum and the metabolic tissue such as thigh muscle. The different feed efficiency (FE) potential of slow-growing chicken is related to duodenal metabolism through proteins enriched in five main metabolic pathways: glycolysis/ gluconeogenesis, peroxisome, oxidative phosphorylation, tight junction, and cysteine and methionine metabolism. The changes in metabolic activity, energy homeostasis, oxidative stress, and tight junction appear to play important roles in regulating FE. The findings suggest that high-FE chickens have better glucose breakdown to extract energy for cellular metabolism from glycolysis and better tight junction strength of their intestinal epithelium than low-FE chickens. On the other hand, low-FE chickens may need to activate their amino acid metabolism and oxidative phosphorylation to provide more compensatory precursors for gluconeogenesis, to prevent disruption in their intestinal barrier function. Since low-FE chickens are more likely to require more energy for maintaining tissue homeostasis and have less usable energy for growth, they gained less weight when consuming the same amount of feed as the high-FE group.

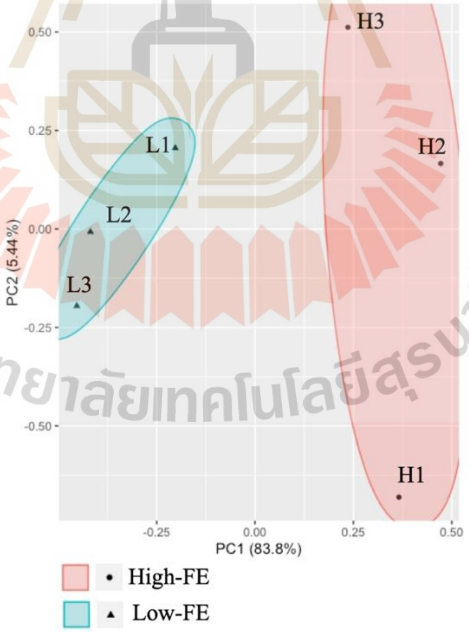
Moreover, our WGCNA results provide evidence that there is an association among the thigh proteomic profile and FE (FCR and RFI) and meat characteristics parameters. This indicated that the changes in both FCR and RFI have an impact on the thigh meat characteristics, flavor, and biomolecules through the alteration of metabolic processes such as glycolysis and gluconeogenesis, metabolic pathway, carbon metabolism, biosynthesis of amino acids, pyruvate metabolism, and protein processing in endoplasmic reticulum (ER). A higher efficiency in high-FE chickens might be due to increased higher rate of glycolytic potential and protein synthesizing potential to generate ATP for protein deposition and maintaining tissue homeostasis.

However, excessive activity in these pathways may result in increased inosine accumulation, altered protein secondary structure, and decreased WHC, indicating that the improvement of FE could potentially have an unfavorable effect on the texture and flavor of meat. Therefore, these findings suggest that using both FCR and RFI as a selection criterion is appropriate for the FE improvement. Moreover, given that the opposite correlation between FE and meat characteristics, selection practices for KR should simultaneously consider both trait groups to maintain the high meat quality of KR while improving FE.

APPENDIX



Supplementary figure 3.1 Principal component analysis (PCA) plot using 355 proteins from Low-FE (blue) and High-FE (red).



Supplementary figure 3.2 Principal component analysis (PCA) plot using 40 DAPs from Low-FE (blue) and High-FE (red).

QR code for Supplementary files

Supplementary Table 3.1. Summary information of mass spectrometry analysis.

Supplementary Table 3.2 Proteins identified by LC-MS / MS analysis in duodenum samples from high- and low-FE of Korat chicken.

Supplementary Table 4.1 Proteins identified in thigh samples from high- and low-FE of Korat chicken.

Supplementary Table 4.2 Proteins identified in the turquoise module

Supplementary Table 4.3 The list of candidate hub proteins related to feed efficiency and meat characteristics parameters

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BIOGRAPHY

Pramin Kaewsatuam was born on August 21, 1994, in Nakhon Ratchasima, Thailand.In 2014, finished high school from Suranaree Wittaya school, Nakhon - Ratchasima. In 2018, she graduated with a bachelor's degree (first honors) from the School of Animal Technology and Innovation at Suranaree University of Technology, Nakhon Ratchasima. Then, she began her Ph.D. program in the School of Animal Technology and Innovation, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, as she received a scholarship from the Royal Golden Jubilee Ph.D. Program (Ph.D. 0198/2558) in Thailand. As part of this scholarship, she spent 1 year in the Department of Agricultural Sciences, Faculty of Agriculture and Forestry, University of Helsinki, Helsinki, Finland, as a visiting student, learning about animal breeding, proteomic analysis, and bioinformatics.

She presented oral and poster presentation including: 1) Poster presented at International Symposium: chicken embryonic stem cells and synchrotron light technology for jumping the S curve of chicken production [2018], Nakhon Ratchasima, Thailand. 2) The 7th SUT International Colloquium on Agricultural Technology, Nakhon Ratchasima, Thailand [2022] Oral presentation on the topic of Comparative proteomics revealed duodenal metabolic function associated with feed efficiency in slow-growing chicken.