TRANSCRIPTOMIC AND METABOLOMIC PROFILE INVOLVED WITH MECHANISM OF CARNOSINE SYNTHESIS IN KORAT CHICKEN

KASARAT PROMKHUN

A Thesis Submitted in Partial Fulfillment of the Requirements for the
Degree of Doctor of Philosophy in Animal Production Technology
Suranaree University of Technology
Academic Year 2023

ทรานสคริปโตมิกส์และเมตาโบโลมิกส์โปรไฟล์ที่เกี่ยวข้องกับกลไกการสังเคราะห์คาร์โนซีนในไก่โคราช

นางสาวกษรัตน์ พรมคุณ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาปรัชญาดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีการผลิตสัตว์
มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2566

TRANSCRIPTOMIC AND METABOLOMIC PROFILE INVOLVED WITH MECHANISM OF CARNOSINE SYNTHESIS IN KORAT CHICKEN

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

Thesis Examining Committee

(Prof. Dr. Pekka Uimari)
Chairperson

(Assoc. Prof. Dr. Amonrat Molee)
Member (Thesis Advisor)

(Dr. Kanjana Thumanu)
Member (Thesis Co-Advisor)

(Assoc. Prof. Dr. Pramote Paengkoum)
Member

(Asst. Prof. Dr. Wittawat Molee)
Member

(Asst. Prof. Dr. Pakanit Kupittayanant)
Member

(Assoc. Prof. Dr. Yupaporn Ruksakulpiwat)
Vice Rector for Academic Affairs and Quality Assurance

(Prof. Dr. Neung Teaumroong)
Dean of Institute of Agricultural Technology

กษรัตน์ พรมคุณ : ทรานสคริปโตมิกส์และเมตาโบโลมิกส์โปรไฟล์ที่เกี่ยวข้องกับกลไกการสังเคราะห์คาร์โนซีนในไก่โคราช (TRANSCRIPTOMIC AND METABOLOMIC PROFILE INVOLVED WITH MECHANISM OF CARNOSINE SYNTHESIS IN KORAT CHICKEN) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร. อมรรัตน์ โมพี, 100 หน้า.

คำสำคัญ: คาร์โนซีน/β-Alanine/L-Histidine/ทรานสคริปโตมิกส์/เมตาโบโลมิกส์/ลำไส้ส่วนเจจูนัม/ไก่กลุ่มโตช้า

ไก่โคราช (KR) ได้รับการพัฒนาเพื่อเป็นทางเลือกสำหรับเกษตรกรรายย่อยในประเทศไทย การเสริมคาร์โนซีนในเนื้อทำให้สามารถแยกไก่โคราชออกจากไก่สายพันธุ์อื่นๆ การสังเคราะห์คาร์โนซีนในไก่สามารถปรับปรุงได้โดยกรดอะมิโนที่เป็นสารตั้งต้นของการสังเคราะห์คาร์โนซีน แต่อย่างไรก็ตามยีนและกลไกทางชีวภาพของการดูดซึมและการขนส่งคาร์โนซีนในไก่โตช้ายังมีการศึกษาเพียงเล็กน้อย ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อตรวจสอบผลของการสังเคราะห์คาร์โนซีนโดยได้จากการเสริมสารตั้งต้นของ β-alanine และ L-histidine ต่อการเปลี่ยนแปลงของทรานสคริปโตมิส์โปรไฟล์ และความสัมพันธ์ระหว่างเมตาโบโลมิกส์โปรไฟล์และสารประกอบทางชีวเคมีในเนื้อเยื่อส่วนเจจูนัมของไก่โคราช ไก่โคราชตัวเมีย 400 ตัว อายุ 21 วัน ถูกแบ่งออกเป็น 4 กลุ่มการทดลอง (กลุ่มละ 5 ซ้ำ/ซ้ำละ 20 ตัว ได้แก่ กลุ่มควบคุม (A) กลุ่มที่เสริมด้วย 1.0% β-alanine (B), 0.5% L-histidine (C) และกลุ่มที่เสริมร่วมกันของ 1.0% β-alanine + 0.5% L-histidine (D) เมื่อไก่อายุ 70 วัน ไก่จะถูกคัดเลือกแบบสุ่มจำนวน 10 ตัวจากแต่ละกลุ่มจะถูกฆ่า และเก็บเนื้อเยื่อลำไส้ส่วนเจจูนัม

เมตาโบโลมิกส์โปรไฟล์จากการสกัดลำไส้เล็กเจจูนัมถูกวิเคราะห์โดยใช้ ^{1}H นิวเคลียร์แมกเนติกเรโซแนนซ์สเปกโทรสโคปี (NMR) มีการระบุสารเมตาบอไลต์ทั้งหมด 28 ชนิด โดยตรวจพบการเปลี่ยนแปลงของความเข้มข้นของสารเหล่านี้ระหว่างกลุ่มอย่างมีนัยสำคัญ Partial least squares discriminant analysis (PLS-DA) สามารถจำแนกสารเมตาบอไลต์ระหว่างกลุ่มทดลองได้ จากสารเมตาบอไลต์พบ metabolic pathways ที่เป็นไปได้ 34 pathways ที่แสดงความแตกต่างระหว่างกลุ่ม และ 8 pathways (โดยมีค่า impact value สูงกว่า 0.05, $P < 0.05$ และ FDR < 0.05) ที่เป็นผลจากปริมาณสารเมตาบอไลท์ นอกจากนี้ยังมีการศึกษาการเปลี่ยนแปลงทางชีวเคมี โดยใช้ synchrotron radiation-based Fourier transform infrared (SR-FTIR) microspectroscopy การเสริม β-alanine เพียงอย่างเดียวในอาหารเพิ่ม β-sheets และลดปริมาณ α-helix และการเสริม L-histidine เพียงอย่างเดียวในอาหารก็เพิ่ม β-sheets เช่นกัน นอกจากนี้ ความสัมพันธ์ระหว่างปริมาณเมตาบอไลท์และสารประกอบทางชีวเคมีได้รับการยืนยันโดยใช้การวิเคราะห์องค์ประกอบหลัก (PCA) ผลลัพธ์จาก PCA ระบุว่ากลุ่ม D มีความสัมพันธ์เชิงบวกอย่างมากกับ amide I, amide II, creatine, tyrosine, valine, isoleucine, และ aspartate การค้นพบนี้สามารถช่วยให้เข้าใจความสัมพันธ์และ

รูปแบบระหว่างกระบวนการทางสเปกตรัมและกระบวนการเมตาบอลิซึมที่เกี่ยวข้องกับการสังเคราะห์คาร์โนซีน

ในการศึกษาทรานสคริปโตมิกส์โปรไฟล์ เนื่องจากเนื้อในกลุ่มที่ได้รับการเสริมด้วยสารตั้งต้นในการสังเคราะห์คาร์โนซีนทั้งสองชนิดจัดเป็นตัวแทนของไก่กลุ่มที่มีคาร์โนซีนสูง เมื่อเปรียบเทียบกับกลุ่มควบคุมที่เป็นตัวแทนของไก่กลุ่มคาร์โนซีนต่ำ โดยตัวอย่างลำไส้เล็กทั้งหมดของไก่โคราช จากกลุ่มควบคุม 5 ตัวและ 5 ตัวได้รับอาหารเสริมด้วยกลุ่ม 1.0% β-alanine + 0.5% L-histidine ผลการวิจัยพบว่า มีการระบุยีนที่แสดงออกแตกต่างกันทั้งหมด 407 ยีน ($P < 0.05$, $\log_2$ fold change ≥ 2) โดยยีน 272 ยีนมีการแสดงออกต่ำ และยีน 135 ยีนมีการแสดงออกสูง ในกลุ่มที่เสริมสารตั้งต้นของการสังเคราะห์คาร์โนซีน เมื่อเปรียบเทียบกับกลุ่มควบคุม การวิเคราะห์ Gene Ontology (GO) แสดงให้เห็นว่ามีการระบุ GO ทั้งหมด 87 GO terms ในลำไส้เล็กส่วนเจจูนัม ($P < 0.05$) การวิเคราะห์ Kyoto Encyclopedia of Genes and Genomes (KEGG) แสดงให้เห็นว่าวิถีทาง KEGG 6 เส้นทางที่มีความสำคัญ ($P < 0.05$) ได้แก่ Neuroactive ligand-receptor interaction, PPAR signaling pathway, Linoleic acid metabolism, MAPK signaling pathway, Calcium signaling pathway และ Arachidonic acid metabolism การวิเคราะห์ลำไส้เล็กส่วนเจจูนัมผ่านการวิเคราะห์ protein-protein interaction network โดยใช้ STRING database ระบุยีน 6 ยีน ได้แก่ *KCND3, OPRM1, CCK, GCG, TRH* และ *GABBR2* ที่ใช้การตรวจสอบรูปแบบการแสดงออกของยีน ผลลัพธ์เหล่านี้ช่วยให้เข้าใจความสัมพันธ์และรูปแบบระหว่างสเปกตรัมและกระบวนการเมแทบอลิซึมที่เกี่ยวข้องกับการสังเคราะห์คาร์โนซีนได้ดีขึ้น และเผยให้เห็นเครื่องหมายโมเลกุลสำหรับอำนวยความสะดวกในการพัฒนาโปรแกรมการคัดเลือกเพื่อปรับปรุงการสังเคราะห์คาร์โนซีนในไก่ที่โตช้า

สาขาวิชาเทคโนโลยีและนวัตกรรมทางสัตว์ ลายมือชื่อนักศึกษา Kansaeit B.
ปีการศึกษา 2566 ลายมือชื่ออาจารย์ที่ปรึกษา
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

KASARAT PROMKHUN : TRANSCRIPTOMIC AND METABOLOMIC PROFILE INVOLVED WITH MECHANISM OF CARNOSINE SYNTHESIS IN KORAT CHICKEN : ASSOC. PROF. AMONRAT MOLEE, Ph. D., 100 PP.

Keyword: CARNOSINE/β-ALANINE/L-HISTIDINE/ TRANSCRIPTOMIC/METABOLOMIC/ JEJUNUM TISSUE/SLOW-GROWING CHICKEN

The Korat chicken (KR) has been developed to provide an alternative breed for smallholder farmers in Thailand. The enrichment of carnosine in meat can distinguish KR from other chicken breeds. Carnosine synthesis in chicken can be improved by amino acids which are substrates of carnosine synthesis. However, there are few studies of the genes and biological pathways of carnosine absorption and transportation in slow-growing chickens. Therefore, this study aimed to investigate the effect of enriched carnosine synthesis, obtained by the β-alanine and L-histidine precursor supplementation, on changes in transcriptomic profiles, and the correlation between metabolomic profiles and biochemical compounds in KR jejunum tissue. Four hundred 21-day-old female KR chickens were divided into 4 experimental groups (5 replicates per group/20 chickens per replicate): a control group (A), dietary supplementation with 1.0% β-alanine (B), 0.5% L-histidine (C), and a mix of 1.0% β-alanine and 0.5% L-histidine (D) group. At 70 days, ten randomly selected chickens from each group were slaughtered and whole jejunum tissues were collected.

The metabolomic profiles of jejunum extraction were analyzed using ^{1}H nuclear magnetic resonance (NMR) spectroscopy. In total, 28 metabolites were identified. Significant changes in the concentrations of these metabolites were detected between the groups. Partial least squares discriminant analysis (PLS-DA) was able to distinguish the metabolites between the experimental groups. Based on the metabolites, 34 potential metabolic pathways showed differentiation between groups and eight pathways (with impact value higher than 0.05, $P < 0.05$, and FDR < 0.05) were affected by metabolite content. Moreover, biochemical changes were monitored using synchrotron radiation-based Fourier transform infrared (SR-FTIR) micro spectroscopy. Supplementation of β-alanine alone in the diet increased β-sheets and decreased α-

helix content and supplementation of L-histidine alone in the diet increased β-sheets as well. Furthermore, the relationship between metabolite contents and biochemical compounds were confirmed using principal component analysis (PCA). Results from PCA indicated that group D was highly positively correlated to amide I, amide II, creatine, tyrosine, valine, isoleucine, and aspartate. These findings can help to understand relationships and patterns between the spectral and metabolic processes related to carnosine synthesis.

Regarding the study of transcriptomic profiles, Owing to the meat in the group that was supplemented with both carnosine synthesis precursors was selected to represent the high-carnosine content group compared with a control group that represented the low-carnosine content group. Whole jejunum samples of KR chicken from 5 control and 5 were fed diet supplemented with 1% β-alanine and 0.5% L-histidine group. The result found that A total of 407 differentially expressed genes ($P < 0.05$, $\log_2$ fold change ≥ 2) were identified, 272 that were down-regulated and 135 that were up-regulated in supplementation of carnosine synthesis precursors, as compared with a control group. The Gene Ontology (GO) analysis showed that a total of 87 GO terms were identified in the jejunum ($P < 0.05$). According to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, 6 pathways were significantly different ($P < 0.05$), Neuroactive ligand-receptor interaction, PPAR signaling pathway, Linoleic acid metabolism, MAPK signaling pathway, Calcium signaling pathway and Arachidonic acid metabolism. The protein-protein interaction network was analyzed using STRING database. The six genes related to Neuroactive ligand-receptor interaction pathway including *KCND3, OPRM1, CCK, GCG, TRH*, and *GABBR2* were performed in the gene expression pattern. These results contribute to a better understanding of relationships and patterns between the spectral and metabolic processes related to carnosine synthesis and reveal the molecular marker for facilitating the development of selection programs for improving carnosine synthesis in slow-growing chickens.

School of Animal Technology and Innovation Student's Signature
Academic Year 2023 Advisor's Signature
Co-advisor's Signature

ACKNOWLEDGEMENT

The success of this work could not have happened without the financial support of the Thailand Research Fund (TRF) and Synchrotron Light Research Institute (Public Organization) through the Royal Golden Jubilee (RGJ) Ph.D. Program (Grant No. PHD/0196/2559) and the Center of Excellence on Technology and Innovation for Korat Chicken Business Development.

First and foremost, I would like to express my sincere gratitude to my thesis advisor, Assoc. Prof. Dr. Amonrat Molee, her exceptional academic expertise, coupled with her commitment to supporting my Ph.D. study and research. Her extensive knowledge, guidance, encouragement, and continuous support throughout the study period have been invaluable.

I would like to express my deepest appreciation to Prof. Dr. Pekka Uimari, Department of Agricultural Sciences, Faculty of Agriculture and Forestry, University of Helsinki, Finland. For giving me an opportunity to attend the courses related to animal breeding at the University of Helsinki. For generously sharing his extensive academic knowledge and playing a pivotal role in helping me establish myself within the academic community. I am truly thankful for his providing suggestions to helpfully improve my article, which not only facilitated significant contributions to publications during my PhD but also opened doors I never thought possible.

I am also grateful to my Co-advisor, Dr. Kanjana Thammanu and my thesis committee members, Assoc. Prof. Dr. Pramote Paengkoum, Asst. Prof. Dr. Pakanit Kupittayanant, and Asst. Prof. Dr. Wittawat Molee, for their constructive comments and beneficial suggestions on my research.

Many thanks to all members of the animal breeding group (especially, Panpradub Sinpru, Rujjira Bunnom, Piyada Chatwanarak, Chanadda Suwanvichanee, and Pramin Kaewsatuan) and all the people at the School of Animal Technology and Innovation during my Ph.D. study at the Suranaree University of Technology for all their kind, friendship, and help.

Words cannot express my gratitude to my beloved family members, especially my mom, Assoc. Prof. Dr. Tussatrin Wannagatesiri, your tireless work and unwavering commitment to providing for me while always emphasizing the value of education have made a profound impact on my journey. My grandparents, you made immense sacrifices and worked tirelessly to provide a better life for me. Look how far we have come! Importantly, I want to express my heartfelt appreciation to my beloved husband, for giving me his true love, patience, and motivation. For his providing me with unfailing support and continuous encouragement through the process of writing this thesis. This accomplishment would not have been possible without them. Last but not least, I wish to express my sincere appreciation to all others who helped me to complete this thesis, but whose names do not appear here.

Kasarat Promkhun

CONTENTS

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF FIGURES (Continued)

Page

LIST OF FIGURES (Continued)

Page

LIST OF ABBREVIATIONS

KR	=	Korat chicken
g/d	=	Gram/day
kg	=	Kilogram
SR-FTIR	=	Synchrotron Radiation Fourier Transform Infrared
mg/g	=	Milligram/gram
EC	=	Enzyme Classification
mRNA	=	Messenger ribonucleic acid
RNA	=	Ribonucleic acid
g	=	Gram
mg	=	Milligram
COOH	=	Carboxyl group
ATP	=	Adenosine triphosphate
FID	=	Independent inductive decay
δ	=	Chemical shift
ppm	=	Parts Per Million
uM	=	Micromolar
mM	=	Millimeter
cm^{-1}	=	Reciprocal centimeter or wavenumber
FDR	=	False Discovery Rate
min	=	Minute
cm	=	Centimeter
mm	=	Millimeter
h	=	hour
°C	=	Degree Celsius
v/v	=	Volume per volume
µL	=	Microliter
mL	=	Milliliter
DSS	=	Sodium trimethylsilyl propane sulfonate

LIST OF ABBREVIATIONS (Continued)

M	=	Molar
bp	=	Base pairs
FC	=	Fold change

CHAPTER I
INTRODUCTION

1.1 Introduction

Korat chicken (KR) is a crossbred chicken between Thai indigenous, Leung Hang Khao sire and a synthetic breed, Suranaree University of Technology (SUT) dam. This establishment aimed to promote the occupation of smallholder farmers in Thailand by creating a uniqueness of the chicken in performance and meat quality. KR is a slow-growing chicken (Poompramun et al., 2021) because the calculations of growth rates are 24 g/d, 2.2-2.3 of feed conversion ratio and average market weight is 1.2 to 1.7 kg at 10 weeks of age (Sinpru et al., 2021). Owing to the low performance of KR, particularly of female KR which has a slower growth rate than male KR, is a significant weak point for farmers. Therefore, adding more value to female KR is desirable and makes smallholder farmers generate more income.

Previous studies reported that carnosine content in female chicken meat is higher than in male chicken meat (Intarapichet and Maikhunthod, 2005; Jung et al., 2013). Therefore, besides improving the growth rate of KR, its competitiveness in the Thai chicken industry can be further improved by increasing the carnosine content in KR meat, given that, in the future, consumers may prefer foods that have favorable effects on health and can make more income for the farmer. Also, the current phase of KR development is the second S-CURVE, which aims to utilize value-added chicken as a natural extract source for pharmaceutical, cosmetic, and nutritional supplement products (Suwanvichanee et al. 2022).

Carnosine which is composed of β–alanine and L–histidine is synthesized by the enzyme carnosine synthase (Mizuno and Kawahara, 2014). It is a cytoplasmic dipeptide found in particularly high concentrations in chicken muscles (Harris et al., 2012; Kai et al., 2015; Kopec et al., 2020; Jukić et al., 2021). Many recent studies have identified the different functions of carnosine, such as the anti-aggregant, antioxidant, and anti-inflammatory activities that are noticeable (Calabrese et al., 2020; Caruso et

al., 2023). The mechanism of carnosine synthesis in chicken can be improved by supplementation of the diet with the carnosine synthesis precursor (i.e., β-alanine and L-histidine) (Cararo et al., 2015; Jukić et al., 2021). Carnosine is easily absorbed from the gastrointestinal tract and enters portal circulation, where it is hydrolyzed into beta-alanine and histidine (Xing et al., 2019). Previous studies reported that carnosine uptake was at its highest in mice jejunum (Ferraris et al. (1988), and in human enterocytes, carnosinase enzyme is high level in the jejunal mucosa (Sadikali et al., 1975).

The small intestine is a highly differentiated and complicated organ, which is not only responsible for the terminal digestion and absorption of nutrients but also plays an important function in amino acid metabolism (Wua et al., 2005). The jejunum is the middle portion of the small intestine, the main function of the jejunum is absorption of important nutrients such as sugars, fatty acids, and amino acids (van Aken, 2010; Denbow, 2012; Simon, 2019). Its also associated with many important physiological processes, such as the regulation of gene expression, synthesis and secretion of hormones, anti-oxidative (Wu, 2009). However, in chicken, the study about carnosine synthesis at a molecular level is limited, it is still unclear how β-alanine and L-histidine can absorb and transported intact across the small intestine, especially in jejunum tissue, and how absorption or transportation is affected by supplementation with carnosine synthesis precursor and also genes and biological pathways that might be related to carnosine synthesis.

Therefore, in this study, we used untargeted metabolomics and Fourier transform infrared (SR-FTIR) microspectroscopy to investigate the metabolic and biochemical changes in the jejunum of KR chickens fed with or without a combination of β-alanine and L-histidine, precursors for carnosine synthesis differentiation, in the diet. Moreover, we would like to study genes and pathways that are related to carnosine synthesis under a diet with and without carnosine synthesis precursors in KR jejunum. of the interplay between metabolites and transcriptome may provide insights into the mechanisms underlying cellular functions and precursor responses in the chicken jejunum.

This study aimed to investigate the metabolic and biochemical compound changes in the jejunum of KR chickens fed with or without a combination of β-

alanine and L-histidine, precursors for carnosine synthesis differentiation, in the diet. Hence, based on the highest carnosine content result from the previous study and the change between metabolic content and biochemical compound, we investigated further the genes and pathways related to absorption and transportation of precursors for carnosine synthesis in jejunum tissue. The knowledge of this study will give an understanding of the interplay between metabolites and transcriptome and may provide insights into the mechanisms underlying molecular functions and a deeper understanding of the related pathways and genes of absorption and transportation of carnosine synthesis precursor leading to the precise find genes marker or biomarkers, and provide confidence to improve the genetic to enhance the ability of carnosine synthesis in KR chicken without adversely impacting the molecular function level.

1.2 Research objectives

The objectives of this study were:

1.2.1 To identify metabolic profiles that related to the effect of enriched carnosine synthesis in Korat chicken jejunum tissue.

1.2.2 To investigate the effect of enriched carnosine synthesis, on changes in biochemical compounds in Korat chicken KR jejunum tissue.

1.2.3 To study the relationship between metabolomic profiles and biochemical compounds in Korat chicken KR jejunum tissue.

1.2.4 To identify differential expression of genes and pathways related to carnosine synthesis in Korat chicken jejunum tissue.

1.3 Research hypotheses

1.3.1 Found the difference between the control group and the β-alanine and/or L-histidine precursor groups of metabolite and metabolic pathways that are associated with carnosine synthesis such as β-alanine metabolism and L-histidine metabolism in the jejunum tissue.

1.3.2 Found the biochemical compounds (i.e., amide I, amide II, CH-bending, amide III, and glycogen/carbohydrate) and the secondary structure ratio (α-helix, β-

sheet, β-turn and antiparallel) changes in the jejunum tissue between the control group and the β-alanine and/or L-histidine precursor groups.

1.3.3 Found the correlation between the metabolite parameter (with VIP score > 1) and biochemical compounds (amide I, amide II, CH-bending, amide III, and glycogen/carbohydrate) and the secondary structure ratio (α-helix, β-sheet, β-turn, and antiparallel) between the control group and the β-alanine and/or L-histidine precursor groups using principal component analysis (PCA).

1.3.4 Found the genes and pathways related to the regulation of β-alanine and L-histidine absorption and/or transportation in Korat chicken jejunum between the control group and the β-alanine and L-histidine precursor groups.

1.4 Scope of the study

At the age of 21 days, the chickens were divided into four experimental groups: a group with a basal diet, a group with a basal diet supplemented with 1.0% (1 g/100 g diet) β-alanine, a group with a basal diet supplemented with 0.5% (0.5 g/100 g diet) L-histidine, and a group with a basal diet supplemented with 1.0% of β-alanine and 0.5% L-histidine. Each experimental group was divided into five replicates with 20 chickens. At 70 days of age, 10 randomly selected chickens from each experimental group were tagged, stunned by chloroform, and exsanguinated by cutting the jugular vein. In the present study, we measured breast meat characteristics traits that are related to the carnosine content, anserine content, TBARS, biochemicals compound, growth performance, meat quality and proteomic profile.

In this study, we collected five to ten pieces of 2 cm long whole jejunum segments. Then, the whole jejunum was cut into 3–5 mm thick pieces, washed in a normal saline solution, and stored in 10% buffered formaldehyde. Afterward, the samples were refrigerated for 24 hours and transferred to a medium containing 80% ethanol until they were embedded in paraffin wax. For SR-FTIR microspectroscopy measurements. The biochemical compounds (i.e., amide I, amide II, CH-bending, amide III, and glycogen/carbohydrate) and the secondary structure ratio (α-helix, β-sheet, β-turn and antiparallel) were obtained.

In addition, a small amount of the whole jejunum was collected, immediately frozen in liquid nitrogen, and stored at −80 ℃ for metabolomic. The metabolomic analysis was performed using proton nuclear magnetic resonance (NMR) spectroscopy, the metabolites were determined manually based on chemical shifts and metabolic pathway analyses were conducted using the MatoboAnalyst 5.0 platform. Moreover, the correlation between metabolite, biochemical compounds and the secondary structure ratio was performed using principal component analysis (PCA).

At the same time, chickens at 10 weeks of age were slaughtered, and 10 whole jejunal tissues were extracted for transcriptome analysis. Then, bioinformatic analysis, i.e., gene ontology (GO), KEGG pathway, and protein-protein interaction network, were used to clarify the differential gene expression and pathway that related to amino acid absorption and transportation in chicken jejunum.

1.5 References

Calabrese, V., Scuto, M., Salinaro, A. T., Dionisio, G., Modafferi, S., Ontario, M. L., Peters, V. (2020). Hydrogen sulfide and carnosine: modulation of oxidative stress and inflammation in kidney and brain axis. **Antioxidants.** 9(12):1303.

Caruso, G., Di Pietro, L., Cardaci, V., Maugeri, S., and Caraci, F. (2023). The therapeutic potential of carnosine: Focus on cellular and molecular mechanisms. **Current Research in Pharmacology and Drug Discovery**. 100153.

Ferraris, R. P., Diamond, J. A. R. E. D., and Kwan, W. W. (1988). Dietary regulation of intestinal transport of the dipeptide carnosine. **American Journal of Physiology-Gastrointestinal and Liver Physiology.** 255(2): G143-G150.

Harris, R. C., Wise, J. A., Price, K. A., Kim, H. J., Kim, C. K., and Sale, C. (2012). Determinants of muscle carnosine content. **Amino acids**. 43(1): 5-12.

Jung, S., Bae, Y. S., Kim, H. J., Jayasena, D. D., Lee, J. H., Park, H. B., and Jo, C. (2013). Carnosine, anserine, creatine, and inosine 5′-monophosphate contents in breast and thigh meats from 5 lines of Korean native chicken. **Poultry Science**. 92(12): 3275-3282.

Jukić, I., Kolobarić, N., Stupin, A., Matić, A., Kozina, N., Mihaljević, Z., and Drenjančević, I. (2021). Carnosine, small but mighty—Prospect of use as functional ingredient for functional food formulation. **Antioxidants**. 10(7):1037.

Kai, S., Watanabe, G., Kubota, M., Kadowaki, M., and Fujimura, S. (2015). Effect of dietary histidine on contents of carnosine and anserine in muscles of broilers. **Animal Science Journal**. 86(5): 541-546.

Kopec, W., Jamroz, D., Wiliczkiewicz, A., Biazik, E., Pudlo, A., Korzeniowska, M., and Skiba, T. (2020). Antioxidative characteristics of chicken breast meat and blood after diet supplementation with carnosine, L-histidine, and β-alanine. **Antioxidants**. 9(11):1093.

Maikhunthod, B. and K. O. Intarapichet. (2005). Heat and ultrafiltration extraction of broiler meat carnosine and its antioxidant activity. **Meat Science**. 71:364-374

Mizuno, D., & Kawahara, M. (2014). Carnosine: A possible drug for vascular dementia. **International Journal of Vascular Medicine**. Surg:2(3).

Poompramun, C., Molee, W., Thumanu, K., and Molee, A. (2021). The significant influence of residual feed intake on flavor precursors and biomolecules in slow-growing Korat chicken meat. **Animal Bioscience**. 34(10):1684.

Sadikali, F., Darwish, R., and Watson, W. C. (1975). Carnosinase activity of human gastrointestinal mucosa. **Gut.** 16(8):585-589.

Simon, Á., Gulyás, G., Mészár, Z., Bhide, M., Oláh, J., Bai, P., and Czeglédi, L. (2019). Proteomics alterations in chicken jejunum caused by 24 h fasting. **PeerJ**. 7: e6588.

Sinpru, P., Riou, C., Kubota, S., Poompramun, C., Molee, W., and A. Molee. 2021. Jejunal transcriptomic profiling for differences in feed conversion ratio in slow-growing chickens. **Animals**. 11(9):2606.

Suwanvichanee, C., Sinpru, P., Promkhun, K., Kubota, S., Riou, C., Molee, W., and Molee, A. (2022). Effects of β-alanine and L-histidine supplementation on carnosine contents in and quality and secondary structure of proteins in slow-growing Korat chicken meat. **Poultry Science**. 101(5):101776.

van Aken, G. A. (2010). Relating food emulsion structure and composition to the way it is processed in the gastrointestinal tract and physiological responses: what are the opportunities?. **Food Biophysics**. 5(4):258-283.

Wu, G. (2009). Amino acids: metabolism, functions, and nutrition. **Amino acids**. 37(1): 1-17.

Wua, G., Knabea, D. A., and Flynnb, N. E. (2005). 5 Amino acid metabolism in the small intestine: biochemical bases and nutritional significance1. **Biology of Metabolism in Growing Animals: Biology of Growing Animals Series**. 3:107.

Xing, L., Chee, M. E., Zhang, H., Zhang, W., and Mine, Y. (2019). Carnosine-A natural bioactive dipeptide: Bioaccessibility, bioavailability and health benefits. **Journal of Food Bioactives**. 5:8-17.

CHAPTER II

LITERATURE REVIEWS

2.1 Korat Chicken (KR)

Korat chicken (KR) is produced from Thai indigenous, Leung Hang Khao sire and synthetic breed, Suranaree University of Technology (SUT) dam. The goal of KR establishment is to get a sustainable carrier for small- to moderate-sized holder farmers in Thailand. KR is a slow-growing chicken (Poompramun et al., 2021) because its growth rate is 24 g/d, feed conversion ratio is around 2.2-2.3 and average market weight is 1.2 to 1.7 kg at 10 weeks of age (Sinpru et al., 2021). As we know the characteristics of KR meat are low fat, high protein, and good texture (Katemala et al., 2021) and can improve meat quality and increase carnosine content when fed with dietary supplementation with β-alanine and L-histidine (Suwanvichanee et al., 2022). However, the lower performance of KR, particularly of female KR, is a significant weak point. In addition to enhancing the growth performance of KR, increasing the biochemical compounds, and improving meat quality at the molecular level can further enhance the competitiveness of the Thai chicken industry. Consequently, adding more value to female KR is desirable

2.2 The situation of Food Consumption and Elderly Population in Thailand

A portion of healthy food is not only popular for the elderly population but also for all ages. Functional food has been accepted by researchers as food which is sensory functions and provides nutritive functions that are also valuable, non-nutritive physical functions of the body such as the immune system, and improves system and body function.

Nowadays, Thailand's aging population has been increasing in proportion to the older population (more than 60 years) over the last 50 years. The proportion of Thai youth and the working-age population has been steadily declining from 2010 to 2040,

while the elderly segment shows a consistent increase from 13.2 percent in 2010 to 32.1 percent in 2040 (Pramote,2016). Interestingly, projections indicate that the youth population is expected to equal the elderly population by 2017 (Figure 2.1).

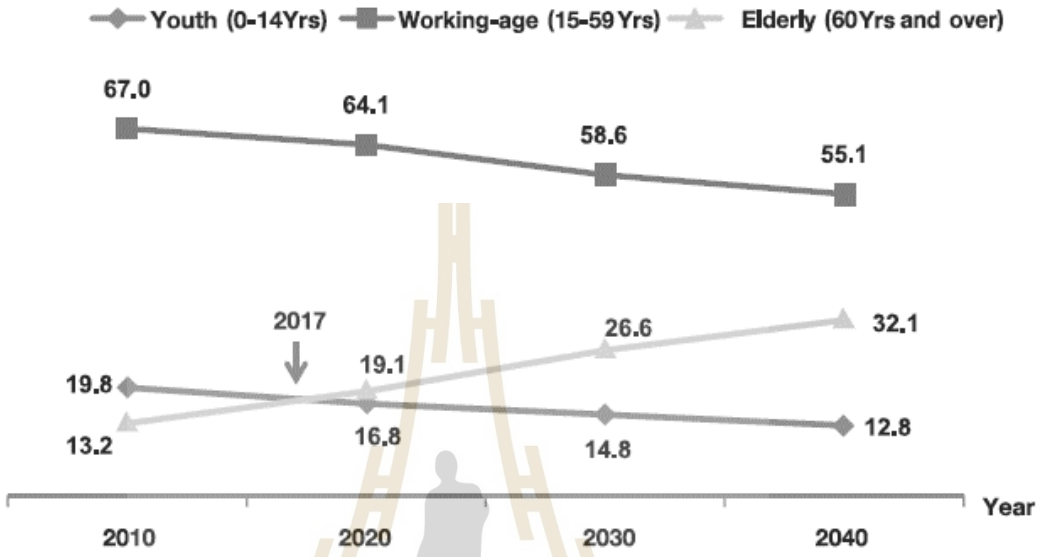

Figure 2.1 The distribution of the population across different age groups-youth, working-age, and elderly-is projected from 2010 to 2040.

Source: Office of the National Economic and Social Development Board.

This situation will affect the demand for food safety and security. The elderly will reduce the amount of food intake because of physical and mental changes. However, the elderly will be more interested in healthy food and food safety. Moreover, people are more interested in the effects of natural health food. Therefore, it is an opportunity to upgrade the existing food value to a higher-value market of products by improving the quality of biomolecules in food for the benefit of elderly health and the entire population.

2.3 Carnosine Structure, Metabolism, and The Relative Compound

Carnosine is a water-soluble dipeptide consisting of β-alanine and L-histidine (Xing et al., 2019). It has several derivatives, among which the methylated analogs anserine and ophidine/balenine are the most prevalent. Carnosine plays an

important role in physiological functions, such as a potent intracellular pH buffer, free radical scavenging, anti-glycation and anti-lipid peroxidation activities (Intarapichet and Maikhunthod, 2005; Xing et al., 2019). Carnosine, anserine, and ophidine are collectively referred to as histidine-containing dipeptides (HCDs) (Blancquaert et al., 2016) and another HCD is homocarnosine which is primarily found in brain structures and arises from the substitution of β-alanine, synthesized from γ-aminobutyric acid (GABA) as shown in Figure 2.2. It is primarily found in mammalian skeletal muscle and neuronal tissue, with smaller amounts present in the heart, liver, and kidney (Boldyrev et al., 2013).

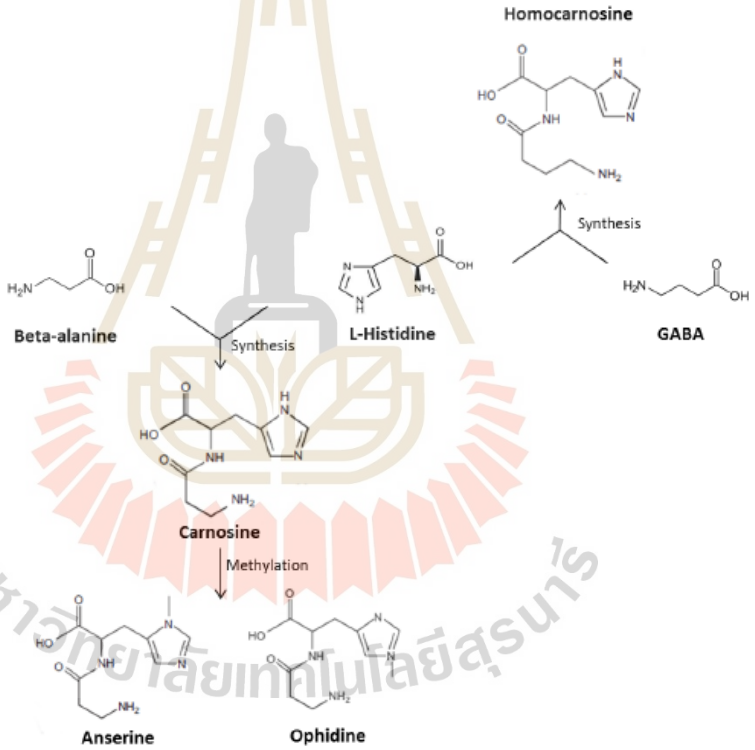

Figure 2.2 Chemical structures of carnosine and its derivatives.
Source: Blancquaert et al. (2016).

Carnosine concentration in each tissue is different, depending on many factors. The content of carnosine can be influenced by various factors including the type of muscle tissue (white or dark meat) and the species of the animal (such as cattle,

sheep, rabbits, and poultry), as well as by factors such as breed, sex, age, and dietary supplementation (Budzen and Rymaszewska, 2013; Boldyrev et al., 2013).

For absorption, uptake, and distribution of carnosine it can be broken down by carnosinase (Bellia et al., 2014) which is present in many tissues such as the kidney, liver, and plasma (Begum et al., 2005). Carnosine levels are regulated by the activity of carnosine synthetase and carnosinase enzymes. Additionally, carnosine can undergo metabolism to form different derivatives, such as anserine and ophidine through methylation, and to acetyl-carnosine through acetylation. The general carnosine metabolism pathway is shown in Figure 2.3.

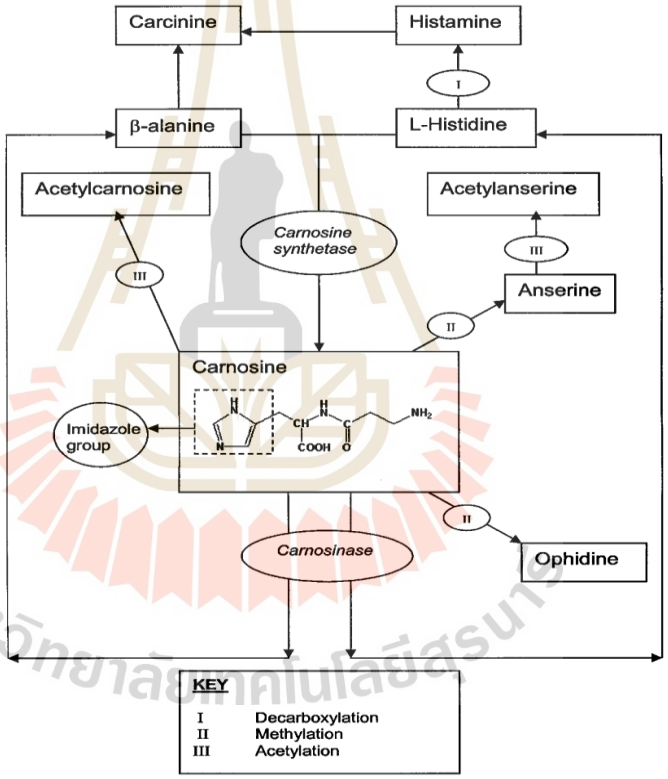

Figure 2.3 Carnosine Metabolism Pathway.

Source: Begum et al. (2005).

2.4 Carnosine Content in Meat

Generally, carnosine is highly abundant in animal muscle. However, its content may base on type of breed, gender, breeding activity, and age (Budzen and Rymaszewska, 2013). Carnosine content in animals of various species is summarized

in Table 2.1. The difference in muscle carnosine content in the body is due to the difference in muscle mass (Decker and Mei, 1996). The carnosine levels in the fast-twitch muscles are higher than in slow-twitch muscles (Culbertson et al., 2010). A previous also found that a high source of carnosine and anserine is a chicken breast extract. It is obtained, anserine content is ~1.4 g/100 mL, and carnosine content is ~0.6 g/100 mL (Currell et al., 2011). However, the different amounts of carnosine in each animal are also influenced by several factors.

Table 2.1 Carnosine content in animals.

Species	Muscle type	Concentration of carnosine (mg/g)	Reference
Beef	Femoris	1.50	Boldyrev and Severin (1990)
	M.Semimembranosus	3.79	Chan et al. (1993)
		4.10	Purchas et al. (2004)
	Rump	7.04	Jones et al. (2011)
	leg	4.43	Mori et al. (2015)
	Shoulder	4.58	
Pork	Shoulder and legs	1.12	Carnegie et al. (1983)
	Semimembranosus	4.19[a]	Mora et al. (2008)
	Biceps femoris	3.68[b]	
	Gluteus maximus	3.26[c]	
	Longissimus dorsi	3.24[c]	
	Gluteus medius	2.59[d]	
	Trapezius	2.10[e]	*Different letters in superscript within the same column mean significant differences ($p < 0.05$).
	Masseter	0.13[f]	
	Rump	5.54	Jones et al. (2011)
	Leg	4.69	Mori et al. (2015)
	Tongue	1.04	
Chicken	Breast	2.80	Boldyrev and Severin (1990)
	Breast	2.90	Maikhunthod and Intarapichet (2005)
	Pactoral	0.54	Jones et al. (2011)
	Breast	2.55	Mori et al. (2015)
	Leg	1.06	
	Fillet	1.57	
Duck	Breast	0.16	Lee et al. (2015)
Lamp	Rump	0.70	Jones et al. (2011)
	Leg	1.22	Mori et al. (2015)
	Shoulder	0.93	
Turkey	Pactoral	0.47	Jones et al. (2011)
	Breast	0.88	Peiretti et al. (2012)

2.5 Factors that Affect Carnosine Content in Meat

The content of carnosine can be influenced by a variety of factors including the type of muscle tissue (white or dark meat) and the species of the animal (such as cattle, sheep, rabbits, and poultry). Additionally, factors such as breed (autochthonous breeds or hybrids), sex, age, breeding, feed, exercise, and stress that summarized in Table 2.2.

Table 2.2 Factors on carnosine content.

Factor	Describe	Reference
Genetic	Breed and line are different	Maikhunthod and Intarapichet (2005), Jung et al. (2013), Kojima et al. (2014)
Species	The content was in increasing order: beef > rabbit > pork > horse > chicken > turkey.	Peiretti et al. (2012)
Type of muscle	fast-twitch muscle is higher than slow-twitch muscle and White muscles higher then red muscles	Culbertson et al. (2010), Tian et al. (2007), Kim et al. (2012), Cornet and Bousset (1999)
Gender	Female higher than male	Everaert et al. (2013)
Age	There is a decrease in carnosine concentration with age	Kralik et al. (2015), Everaert et al. (2013), Kim et al. (2012), Jung et al. (2013)
Diet	Organisms on a vegetarian diet have a lower concentration of carnosine in the skeletal muscles and L-histidine supplementation > β-alanine supplementation	Tomonaga et al. (2012), Kai et al. (2015), Kralik et al. (2015), Derave et al. (2010), Cong et al. (2017)
Exercise	Trained muscle is higher than Untrained muscle	Bex et al. (2013)

2.6 Carnosine Synthesis and Degradation

ATP-dependent carnosine synthase enzyme (EC 6.3.2.11) synthesizes carnosine from β-alanine and L-histidine as shown in Figure 2.4. It is detected in the extracts of chicken muscle and mouse brain (Cararo et al., 2015). Carnosine synthase activity is detected in neuroglia derived from the rat brain (Bellia et al., 2014) and catalyzes the synthesis of homocarnosine (Drozak et al., 2010).

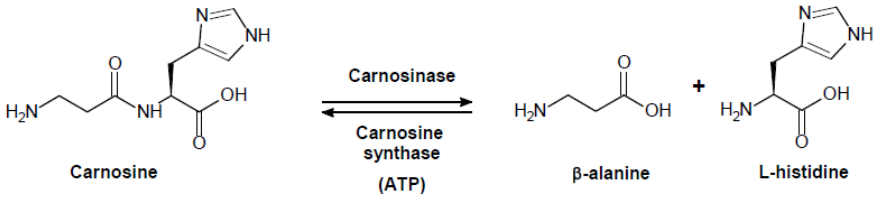

Figure 2.4. Chemical Structures of β-alanine, L-histidine, and Carnosine.
Source: Varanoske et al. (2019).

The carnosine metabolism pathway engages multiple enzymes and transporters. The primary pathways involved in the regulation of tissue carnosine content include synthesis from precursor amino acids, catalyzed by carnosine synthase (CARNS), and hydrolysis into its constituents by carnosinase. Carnosinase is further categorized into serum carnosinase (CN1) and tissue carnosinase (CN2) (Lenney et al., 1985).

Supplementation and food sources increasing the dietary intake of carnosine enhance its concentrations in, mostly, skeletal muscle. In the serum and tissue, carnosine is released by carnosinase enzyme. CN1 is highly active in human blood (Lenney et al., 1985). It is responsible for most of the carnosine degradation (Derave et al., 2010). Whereas, CN2 is present in many tissues such as liver, kidney, and small intestine (Lenney et al., 1985). It is involved in carnosine degradation in tissues with abundant carnosine content and the intestinal wall (Boldyrev et al., 2013) but the activity of CN2 is rather low (Sadikali et al., 1975). A part of the ingested carnosine likely enters the bloodstream intact where it can hydrolyze rapidly in plasma due to the high activity of CN1 as shown in Figure 2.4. The constituent amino acids β-alanine and L-histidine are transported into muscle cells by specific amino acid transporters. Once inside the cell, carnosine synthase (CARNS) catalyzes the synthesis of carnosine from β-alanine and L-histidine (Drozak et al., 2010; Perim et al., 2019). The genes related to carnosinase are carnosinase dipeptidase 1 *(CNDP1)* and carnosinase 2 *(CNDP2).* It is in human chromosome 18 in a head-to-tail position (McDonough et al., 2009).

Thus, the highest rate of CARNS activity is found in mammalian skeletal muscle tissue (Abe, 2000) and the enzyme is highly expressed on mRNA level in human

skeletal muscle (Everaert et al., 2013). Carnosine along with mRNA expression of CARNS is also recently discovered in liver, kidney, retina, and spleen rat tissues but its concentration is lower than in muscle (Boldyrev et al., 2013) as shown in Table 2.3

Table 2.3 The presence of carnosine metabolism enzymes in tissues.

Rodent	Blood	Skeletal muscle & heart	Brain/CNS	Other organs	
CARNS	-	**	**	** kidney Liver Ratina	** Spleen
CN1	-	-	-	* Small intestine	** Kidney
CN2	-	*	*	* Liver	** Small intestine Kidney
Human	**Blood**	**Skeletal muscle & heart**	**Brain/CNS**	**Other organs**	
CARNS	-	**	**	* Kidney	
CN1	**	-	*	* Liver Kidney	
CN2	-	*	*	* Lung spleen liver	** Kidney Small intestine

- Absent, * little present, ** abundantly present. CN1: serum carnosinase; CN2: tissue carnosinase, CARNS: carnosine synthase; CNS: central nervous system. Apply from Kamal et al. (2009); Mong et al. (2011); Pfister et al. (2011); and Riedl et al. (2011).

Therefore, the expression of CARNS was also recently detected in liver, kidney, retina, and spleen tissues and is highly present in muscle and brain of rat. CN1 is abundant and present in rodent kidneys and human blood. CN2 has been demonstrated in the kidney, liver, spleen, and small intestine.

2.7 β-alanine Pathways and Metabolism

β-alanine is a non-essential amino acid and it is also one of the precursors to carnosine, along with L-histidine (Culbertson et al., 2010). It is a naturally occurring free β-amino acid, or non-proteinogenic (Hoffman et al., 2015). β-alanine synthesis pathways have three steps in the liver-derived from pyrimidines thymine degradation, cytosine degradation, and uracil degradation and its transport to skeletal muscle by sodium- and chloride-dependent (Zanella et al., 2016).

β-alanine synthesis pathways include uracil degradation and aspartate decarboxylation by the glutamate decarboxylase-like protein 1 (GADL1) enzyme, and pathways for β-alanine degradation by GABA transaminase enzymes (GABA-T) and Alanine-Glyoxylate Aminotransferase 2 (AGXT2) (Blancquaert et al., 2016).

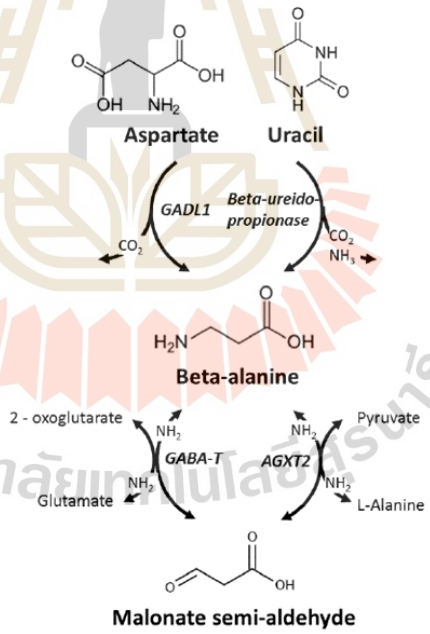

Figure 2.5 β-alanine Synthesis and Degradation Pathways.
Source: Blancquaert et al. (2016).

Several transporters that can transfer β-alanine are known as Proton-coupled amino acid transporter 1 (*PAT1*), Taurine transporter (*TauT*) and ATB $^{0,+}$ (Blancquaert,

2017). The amine group of β-alanine can be removed through a transamination process, leading to the formation of the keto-acid malonate semi-aldehyde (MSA). This MSA can then enter the citric acid cycle, also known as the Krebs cycle, where it can contribute to energy production. The enzymes 4-aminobutyrate-2-oxoglutarate transaminase (GABA-T or β-alanine-2-oxoglutarate transaminase) and alanine-glyoxylate transaminase (AGXT2 or β-alanine-pyruvate transaminase) are both enzymes to catalyze this reaction as shown in Figure 2.5. GABA-T and AGXT2 are enzymes that can transaminase β-alanine in mammals. It is capable of synthesizing β-alanine from malonate semi-aldehyde (Blancquaert et al., 2016).

2.8 β-alanine Pathways and Metabolism

L-histidine is a proteinogenic α-amino acid (essential amino acid). The major pathways of L-histidine metabolism are chemical and biological properties such as proton buffering, metal ion chelation, and antioxidant functions (Holeček, 2020).

L-histidine is the secondary precursor of the dipeptide for carnosine synthesis (Blancquaert et al., 2016) and the synthesis of histamine by removal of the COOH group of histidine by the enzyme histidine decarboxylase (Tanaka and Ichikawa, 2006). Two proteins, peptide/histidine transporter 1 and 2 (PHT1 and PHT2) are groups of the proton-coupled oligopeptide transporters (POT-family or SLC15) and POT family are oligopeptide transporter 1 and 2 (PEPT1 and PEPT2).

The overall pathways of L-histidine metabolism as shown in Figure 2.6 synthesis of L-histidine turnover and protein breakdown and L-histidine catabolism through urocanate to glutamate. The main pathway of L-histidine catabolism can catalyze the formation of histamine from L-histidine by histidase (EC 4.3.1.3), the rate-limiting enzyme of histidine degradation, leading to the production of trans-urocanate and ammonia. The enzyme is primarily located in the epidermis and liver (Holeček, 2020).

In the liver, found that histidase expression is regulated by L-histidine availability. It will increase when protein intake is high. Urocanase (EC 4.2.1.49) enzymes can hydrolyze the urocanate produced to form iminoglutamate (FIGLU) by a histidase reaction to imidazolone propionic acid. FIGLU is converted to glutamic

acid by formimino transferase (E.C. 2.1.2.5) (Holeček, 2020). Tetrahydrofolate (THF)-dependent reaction is sourced from various origins, potentially influencing the flux of L-histidine through the L-histidine degradation pathway. Glutamate is generated by the reaction of formimino transferase which can then be utilized for the glutamine synthesis.

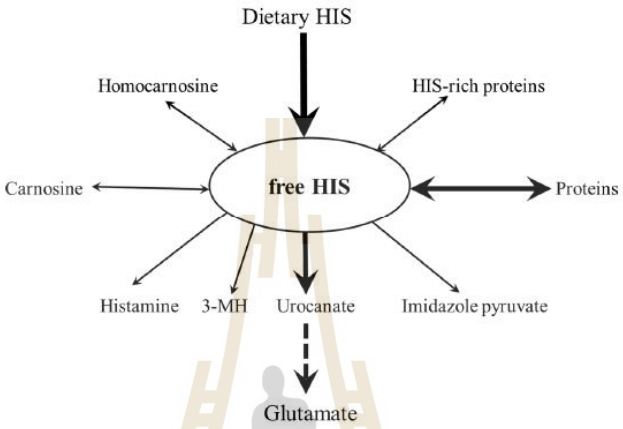

Figure 2.6 L-histidine Metabolism.
Source: Holeček (2020).

2.9 Influences of β-Alanine and L-Histidine Supplementation

Carnosine is synthesized by hydrolysis of ATP from L-histidine and β-alanine in skeletal muscle and other tissues, such as brain and heart (Artioli et al., 2010). The main pathways involved in carnosine metabolism are synthesis by carnosine synthase (CARNS) (Drozak et al., 2010) and hydrolysis to its constituent amino acids by carnosinase (Teufel et al., 2003) as shown in Figure 2.6.

β-alanine is an amino acid that can be produced in liver and can also be found in animal proteins. β-alanine supplementation has been shown to have a beneficial effect on increasing muscle carnosine levels, thereby improving exercise performance. The primary physiological mechanism behind this effect is the ability of β-alanine to combine with another amino acid, particularly L-histidine, within tissues such as the brain and skeletal muscle (Varanoske et al., 2019). The limited rate of enzymatic condensation reaction occurs because of the β-alanine availability

(Derave et al., 2010). β-alanine can be synthesized from many pathways including the synthesis from aspartate amino acids, the conversion from α-alanine form, the metabolism of acryloyl-CoA, or from oxaloacetic acid (Wang et al., 2013). Although β-alanine could also be synthesized from the degradation of uracil, there is no report on the relation between carnosine synthesis and pyrimidine catabolism (Takayuki et al., 2012).

Carnosine can be obtained through the diet and hydrolyzed by the carnosinase enzyme in the gastrointestinal tract. Carnosine cannot be uptake from the bloodstream (Artioli et al., 2010) (Figure 2.6). Moreover, L-histidine or β-alanine is not produced in skeletal muscle, whose production is limited to the liver cells (Sale et al., 2010). Therefore, carnosine is primarily dependent on the absorption of β-alanine and L-histidine by endogenous synthesis in the muscle cells (Caruso et al., 2012).

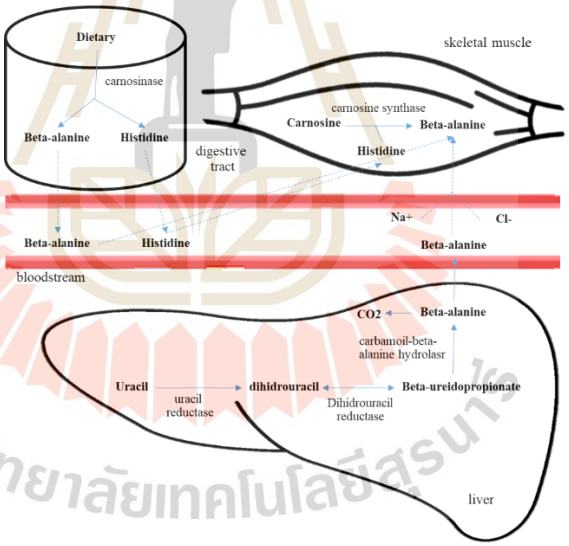

Figure 2.7 Endogenous synthesis of β-alanine by the liver, absorption of dietary carnosine by the digestive tract, and β-alanine uptake of carnosine synthesis by skeletal muscle.
Source: Artioli et al. (2010).

2.10 Gene Expression of Carnosine-Related Enzymes and Transporters

Carnosine intake primarily relies on the meat protein absorption of through the gastrointestinal tract. The absorption of carnosine is predominantly facilitated by proton-coupled peptide transporters (PepT) located in the small intestine (Son et al., 2004) as shown in Figure 2.8. The ingestion, transport, and synthesis of carnosine in muscles have been found in many studies on humans and mice. After consumption, carnosine is absorbed through the epithelium cell in the small intestine by oligopeptide transporters 1 and 2 (PepT1 and PepT2) on the brush border membrane, it is expressed in the small intestine, renal proximal tubule, and liver, facilitating the uptake of carnosine. The competitive uptake is observed when other dipeptides are present in the transport solution, while the inclusion of amino acids does not significantly affect the uptake of carnosine. Once absorbed, carnosine encounters carnosinase enzymes, which can break down into β-alanine and L-histidine in the small intestine. These breakdown products can then be transported into the bloodstream by amino acid transporters. Only a small portion of carnosine is absorbed into the bloodstream unchanged via peptide transporters (Boldyrev et al., 2013). Therefore, both amino acids are transported by amino transporters into sarcoplasm muscle and synthesized by carnosine synthase into carnosine (Harris et al., 2012).

While β-alanine can be synthesized from the body through many pathways in the liver and transmitted via the bloodstream. The carnosine synthase in muscle will be released for binding β-alanine and histidine into carnosine, independently. Homocarnosine can combined with L-histidine and gamma-aminobutyric acid (GABA) with an enzyme that has the same characteristics as carnosine. In the path of anserine and opine are created by specific enzymes, namely carnosine methyltransferase (EC 2.1.1.22) (Anderson et al., 2009).

Carnosine can accumulate to some extent within renal epithelial cells and undergoes degradation by carnosinase 2 (CN2), resulting in the release of β-alanine and histidine. These released components then pass across the basolateral membrane via amino acid transporters. Subsequently, β-alanine and histidine are reassembled within the tissue through the enzymatic activity of carnosine synthase, as shown in Figure 2.8. The proton-dependent uptake of carnosine is mediated by the human peptide/histidine transporter 1 (hPHT1) (Bhardwaj et al., 2006). Carnosine

in the serum or tissue is degraded primarily by carnosinase enzymes. In humans, CN2 is almost completely absent from skeletal muscle tissue. Therefore, CN1 isresponsible for most of the carnosine degradation (Derave et al., 2010).

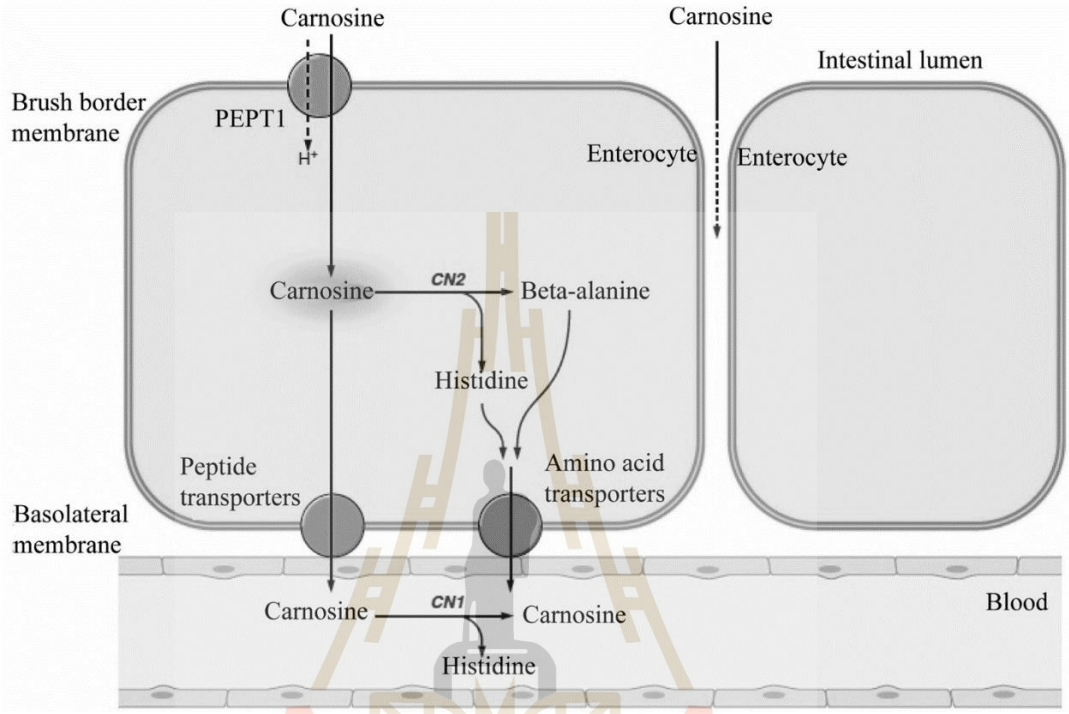

Figure 2.8 Carnosine Uptake Mechanisms in Enterocytes.
Source: Boldyrev et al. (2013).

Functional groups within carnosine molecules are essential for their recognition by carnosinase and carnosine transporters as shown in Figure 2.9.

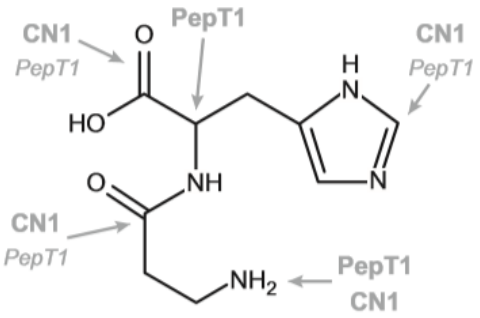

Figure 2.9 Carnosine Derivatives Structure.

Source: Boldyrev et al. (2013).

Through mRNA and protein analysis of gut sections and protein lysates extracted from the animal gastrointestinal tract, it was discovered that Carnosine/ histidine transporters 1 (PHT1) are predominantly expressed in the epithelial cells of the small intestine. However, knowledge of the transport of carnosine through hPHT1 remains limited and warrants further investigation.

Everaert et al. (2013) reported a group of genes that regulated to the synthesis and transport of carnosine in humans and mice (Miyaji et al., 2012). It was found that gene expression was classified into 5 groups according to their roles such as the β-alanine transporters, TauT and PAT1, are related to absorption of β-alanine (Iruloh et al., 2007), Carnosine synthase (CARNS) is associated with carnosine synthesis, Carnosinase-1 and Carnosinase-2 (CNDP1 and CNDP2) associated with carnosine breakdown (Ma et al., 2010), Oligopeptide transporter 1 and 2 (PepT1 and PepT2) and Carnosine/ histidine transporters 1 and 2 (PHT1 and PHT 2) are related to the histidine transport (Agu et al., 2011), β-alanine transaminase (ABAT) is associated with β-alanine transaminase and Histidine decarboxylase (HDC) are related to histamine synthesis (Anderson et al., 2009) and the summarizes of expression of carnosine-related enzyme and transporters as shown in Table 2.4.

Moreover, in swine, was reported CARNS1 gene expression in skeletal muscle (Yang et al., 2014). In chickens, reported that the expression of histamine N-methyltransferase-like protein (HNMT-Like gene) is related to the anserine synthesis of methylation with carnosine-by-carnosine N-methyltransferase and ATP-grasp domain-containing protein 1 (ATPGD1) gene on chromosome W associated with carnosine synthesis (Drozak et al., 2010; Drozak et al., 2013). Moreover, the mRNA expression of CARNS1, CNDP1, CNDP2, SLC6A6, SLC36A1, and SLC6A14 was found in humans and mice, also in pectoral muscle (Qi et al., 2018). Of all the 6 genes, only CNDP1 and SLC6A14 in the breast muscle of chicken could not be detected.

Table 2.4 Gene expression of carnosine-related enzymes and transporters.

Function	Species	Official name	Trivial name	Reference
Carnosine synthesis	Mouse	CARNS1	CARNS1	Ma et al. (2010)
	Human			
	Pig			Yang et al. (2014)
	Chicken	ATPGD1	ATPGD1	Drozak et al. (2010)
Carnosine hydrolysis	Mouse	CNDP1, CNDP2	CNDP1, CNDP2	Ma et al. (2010)
	Human			
Carnosine transport	Mouse	SLC15A1, SLC15A2	PEPT1,	Agu et al. (2011)
	Human		PEPT2	
Carnosine/histidine transport	Mouse	SLC15A4, SLC15A3	PHT1,	Agu et al. (2011)
	Human		PHT2	
β-alanine transport	Mouse	SLC6A6, SLC36A1,	TauT,	Iruloh et al. (2007)
	Human	SLC6A14	PAT1	Anderson et al. (2009)
			$ATB^{0,+}$	
β-alanine transaminase	Mouse	Abat	ABAT	Anderson et al. (2009)
	Human			
Histamine synthesis	Mouse	HDC	HDC	Anderson et al. (2009)
	Human			
Anserine synthesis	Chicken	HNMT-Like	HNMT-Like	Drozak et al. (2013)

Carnosine synthase 1 (CARNS1), Carnosinase-1 and Carnosinase-2 (CNDP1 and CNDP2), Oligopeptide transporter 1 and 2 (PEPT1 and 2) SLC6A6, taurine transporter; SLC36A1, Proton coupling amino acid transporter; SLC6A14, solute carrier family 6 member 14, Carnosine/ histidine transporters 1 and 2 (PHT1 and 2), Beta-alanine transaminase (ABAT), Histidine decarboxylase (HDC), 4-aminobutyrate-2-oxoglutarate transaminase (GABA-T), alanine-glyoxylate transaminase (AGXT2).

2.11 Metabolomic Approach

Metabolomics was used to investigate the dynamic changes of metabolism in meat during storage and under different conditions of processing (Castro-Puyana et al., 2017). This approach is a major theoretical basis to annotate the mechanisms behind meat quality changes and to improve meat product storage technologies (Wen et al., 2020). Successful use of metabolomics in various fields of food science (Li et al., 2021) shows assurance of analysis in meat quality and meat safety control (Zhu et al. al., 2020; Zhang et al., 2021). That allows a brief metabolomics technologies overview, this was followed by a critical review of the latest advances in metabolomics of meat and meat products on quality assessment and authentication.

The most common spectroscopic analysis technique Nucleus Magnetic Resonances (NMR) can simultaneously identify and quantify a wide range of organic compounds over the micromolar range. It provides unbiased information on the metabolite profile (Kim et al., 2021). Increasing the amount of NMR studies used to assess the metabolic profile of meat samples without evaluating the chemistry of the target metabolites (Simmler et al., 2014). NMR combined with multivariate analysis was used to understand and/or clarify metabolic changes in meat (Kim et al., 2021). Several studies have used NMR-based analysis of chicken meat samples to find the metabolic profiles and to differentiate meat by species, age, the origin of meat, and metabolic fingerprints (Beauclercq et al., 2016). Therefore, profiling of these metabolites provides detailed information on the metabolic pathway and also a piece of important information related to the physiological state of living things including useful for discovering biomarkers of meat quality.

NMR is widely used to determine metabolite transformations in meat. NMR spectroscopy is a technique that uses the magnetic properties of protons to obtain information related to molecular structure and identity (Muthubharathi et al., 2021). The samples were placed in a strong magnetic field and electromagnetic radiation in the form of radio-frequency pulses was used to excite the protons as shown in Figure 2.10. When the protons are freed from equilibrium energy is recorded as an oscillating electromagnetic signal. This is called independent inductive decay (FID). Each peak frequency overlaps and decays along the way. This waveform is complex

(intensity vs. time) and usually, Fourier transforms to generate a spectrum (Rankin et al., 2014), which is like the separation of individual frequencies listened to by individual peaks, identifying what those frequencies are and how loud each frequency. The data are expressed as the chemically shifted peak spectra (δ), in parts per million (ppm) along the x-axis and intensity along the y-axis (Rankin et al., 2014).

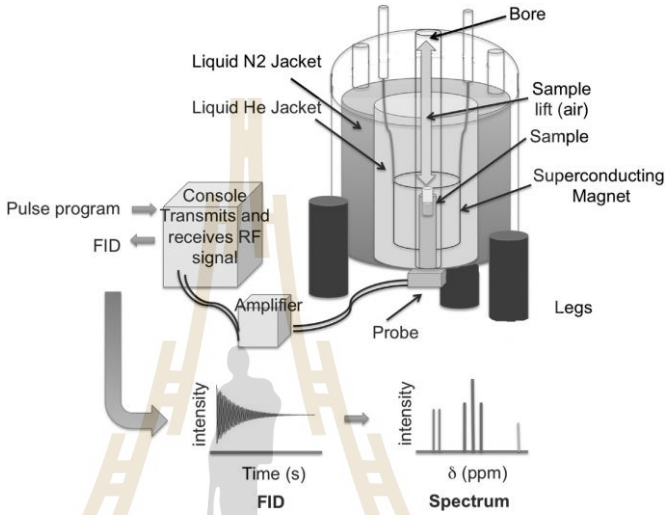

Figure 2.10 Nuclear Magnetic Resonance Spectrometer Diagram.
Source: Rankin et al. (2014).

NMR-based metabolism has a clear advantage for tissue metabolism, whereby ^{1}H-High Resolution Magic Angle Spinning can be directly used in sample analysis (Brennan, 2014). NMR is used in various fields. The steps involved in the NMR-based metabolomics study overview are provided in Figure 2.11.

Nuclear Magnetic Resonance (NMR) remains one of the most frequently used analytical platforms in metabolism (Brennan, 2014). It is often chosen for its absolute quantitative reliability and utility. Because NMR is non-destructive, unbiased, can be easily measured little or no separation is required, allows identification of new compounds, and does not require chemical modification (Wishart, 2013). However, NMR is relatively insensitive and is limited to substances measured in micromolar (uM) to millimeter (mM) concentrations. In general, NMR can detect 50 to 75 compounds in a given human biological fluid, with a lower sensitivity limit of

approximately 5 μM (Brennan, 2014). Most of the compounds detected by NMR spectroscopy are indeed intrinsically polar molecules, including organic acids, sugars, amino acids, and small amines (Wishart, 2013).

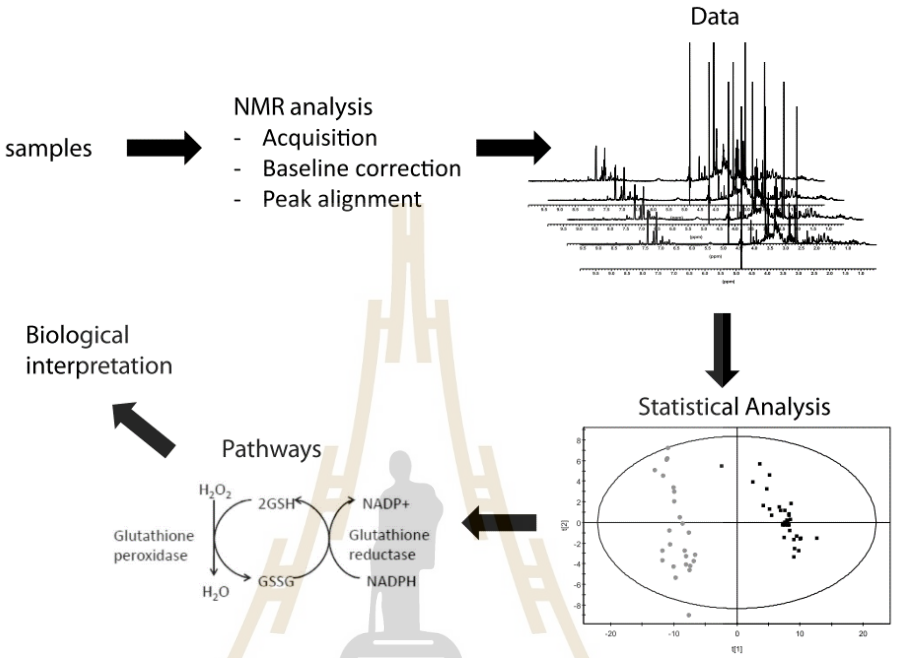

Figure 2.11 Overview of NMR-based Metabolomics Analytical.
Source: Brennan (2014).

NMR-based metabolism provides simultaneous structural and quantitative information. This can be particularly useful in identifying unknown substances, which is a major limitation of metabolomics. Despite these advantages, it must be admitted that NMR metabolism has many limitations. The most important limitation is low sensitivity when compared with the MS-based method (Brennan, 2014).

Therefore, NMR is a stable and reproducible approach and almost every main class of metabolites has a characteristic NMR spectrum. The application of metabolic analysis provides detailed information on metabolism pathways and biological processes. May be helpful for better understanding metabolic changes in meat quality. It also can help in identifying biomarkers that are related to the change in metabolic profile.

2.12 Fourier Transform Infrared (FTIR) Microspectroscopy

Fourier transform infrared microspectroscopy (FTIR) was used to study molecular function groups and can be used for samples qualitative and quantitative (Saputra et al., 2018). this approach is typically associated with differences in the properties and composition of the components. FTIR spectroscopy has proven to be an invaluable tool for extracting unique information about biochemical changes occurring in cells and tissues (Thumanu et al., 2014). The main advantages of FTIR are a non-destructive, rapid, and simple method for identifying the chemical structure of biological molecules and a higher speed of data acquisition. The mid-IR (400-4000 cm^{-1}) is widely employed for analysis due to the presence of characteristic absorbance frequencies and primary molecular vibrations exhibited by all molecules within this spectrum (Davis and Mauer, 2010) such as carbohydrates, nucleic acids, proteins, and lipids.

FTIR is also able to measure the vibration of amino acids and probes the vibrational properties of amino acids and cofactors which are sensitive to structural changes (Berthomieu and Hienerwadel, 2009). Previous studies have found the use of FTIR to analyze the synthesis of L-carnosine and its applications in biomedical fields (Khosravi et al., 2014).

Based on previous studies, infrared spectroscopy can result in the identification of different kinds of samples. In the animal breeding field, there is no study related to the FTIR application. However, this technique can be used to identify the species from processed meat products, detect the authenticity of the species, adulteration of meat or fat from various animals, and compare the chemical composition of meat in each species against other pure samples of different types of animals including pig, chicken, lamb, and beef based on FTIR and principal component analysis techniques (Saputra et al., 2018).

2.13 Transcriptomic Profiling

The study of carnosine synthesis mechanism in chickens has not been widely studied. Previous studies have reported that 5 genes and enzymes are related to

carnosine synthesis (Everaert et al., 2013; Ma et al., 2010; Agu et al., 2011; Anderson et al., 2009) as shown in Table 2.5. and 4 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways including Arginine and proline metabolism metabolic pathways, organism-specific biosystem, Histidine metabolism and β-alanine metabolism (NCBI, http://www. ncbi. nlm. nih. gov).

Table 2.5 Genes involved with carnosine synthesis.

Official Symbol	Official Full Name	Gene ID	Gene type	Location
CARNMT1	carnosine N-methyltransferase 1	427256	protein coding	chromosome: Z
CARNS1	carnosine synthase 1	100359387	protein coding	chromosome: 5
LOC771456	histamine N-methyltransferase-like	771456	protein coding	chromosome: 7
CNDP1	carnosine dipeptidase 1	421012	protein coding	chromosome: 2
CNDP2	CNDP dipeptidase 2 (metallopeptidase M20 family)	421013	protein coding	chromosome: 2

Source: NCBI, http://www. ncbi. nlm. nih. Gov.

In this study, the expression of genes and pathways of carnosine synthesis in chickens will be investigated. Based on the previous studies, it is indicated that carnosine formation catalyzed by carnosine synthase belongs to the "ATP-grasp family" of ligases. A database mining approach identified ATPGD1 in chicken (Drozak et al., 2013). However, the study of all genes encoding carnosine-related enzymes and synthesis will be conducted to provide a better understanding of the biological function of carnosine and its derivatives and to identify genes and pathways related to carnosine synthesis in chickens.

Due to, transcriptomic is the total complement of RNA transcripts in whole cells and organisms including messenger RNA, and non-coding RNA (Kukurba and Montgomery, 2015) caused by the expression of various genes in the genome. Transcriptome profiling is the expression of genes at the same time, as well as, providing gene expression profiles that describe changes in the transcriptome in response to a particular condition or treatment. Gene products within cells have more variety which is different in the expression and modification process of RNA transcript in different cells and conditions. This is one of the important factors that cause a variety of gene expressions. Therefore, a study of the expression of RNA

transcript or transcriptome is very useful for understanding biological function (Wang et al., 2009).

RNA-sequencing or RNA-seq has been widely used in discovering transcriptomic differences in a variety of tissues (Liu et al., 2018) for identifying candidate genes and key pathways. Its approach provides a more accurate quantification of gene expression (Sabino et al., 2018). Nowadays, RNA-Seq has many studies applied to analyze various tissues in chickens including muscle, liver, hypothalamus, and ovarian follicle (Wan et al., 2017). RNA-Seq data is used in different expressed gene identification, and it has also been successfully applied in gene co-expression network analysis (Sabino et al., 2018; Zhang et al., 2019). For diet modification, e.g., consumption of more meat as a source of carnosine may suffice if supplementation is indeed indicated (Jargin, 2016). Therefore, the knowledge of this study can reveal the pathways and genes involving carnosine synthesis in chickens. It plays a key role in gene biomarker selection that can increase carnosine synthesis in chickens in the future.

2.14 References

Abe, H., and Okuma, E. (1995). Discrimination of meat species in processed meat products based on the ratio of histidine dipeptides. **Journal of the Japanese Society for Food Science and Technology.** 42: 827–834.

Agu, R., Cowley, E., Shao, D., MacDonald, C., Kirkpatrick, D., Renton, K., and Massoud, E. (2011). Proton-coupled oligopeptide transporter (POT) family expression in human nasal epithelium and their drug transport potential. **Molecular pharmaceutics.** 8(3): 664-672.

Anderson, C. M., Howard, A., Walters, J. R., Ganapathy, V., and Thwaites, D. T. (2009). Taurine uptake across the human intestinal brush-border membrane is via two transporters: H+-coupled PAT1 (SLC36A1) and Na+-and Cl−-dependent TauT (SLC6A6). **The Journal of physiology**. 587(4): 731-744.

Artioli, G. G., Gualano, B., Smith, A., Stout, J., and Lancha Jr, A. H. (2010). Role of beta-alanine supplementation on muscle carnosine and exercise performance. **Medicine and Science in Sports and Exercise.** 42(6):1162-73.

Begum, G., Cunliffe, A., and Leveritt, M. (2005). Physiological role of carnosine in contracting muscle. **International journal of sport nutrition and exercise metabolism.** 15(5): 493-514.

Bellia, F., Vecchio, G., and Rizzarelli, E. (2014). Carnosinases, their substrates and diseases. **Molecules.** 19(2):2299-2329.

Berthomieu, C., and Hienerwadel, R. (2009). Fourier transform infrared (FTIR) spectroscopy. **Photosynthesis research**. 101(2-3):157-170.

Bex, T., Chung, W., Baguet, A., Stegen, S., Stautemas, J., Achten, E., and Derave, W. (2013). Muscle carnosine loading by beta-alanine supplementation is more pronounced in trained vs. untrained muscles. **Journal of applied physiology.** 116(2): 204-209.

Bhardwaj, R. K., Herrera-Ruiz, D., Eltoukhy, N., Saad, M., and Knipp, G. T. (2006). The functional evaluation of human peptide/histidine transporter 1 (hPHT1) in transiently transfected COS-7 cells. **European journal of pharmaceutical sciences.** 27(5):533-542.

Blancquaert, L., Baba, S. P., Kwiatkowski, S., Stautemas, J., Stegen, S., Barbaresi, S., and Everaert, I. (2016). Carnosine and anserine homeostasis in skeletal muscle and heart is controlled by β-alanine transamination. **The Journal of physiology.** 594(17):4849-4863.

Boldyrev, A. A., and Severin, S. E. (1990). The histidine-containing dipeptides, carnosine and anserine: distribution, properties and biological significance. **Advances in Enzyme Regulation**. 30:175-188.

Boldyrev, A. A., Aldini, G., and Derave, W. (2013). Physiology and pathophysiology of carnosine. **Physiological reviews**. 93(4): 1803-1845.

Brennan, L. (2014). NMR-based metabolomics: From sample preparation to applications in nutrition research. **Progress in nuclear magnetic resonance spectroscopy.** 83:42-49.

Budzeń, S., and Rymaszewska, J. (2013). The biological role of carnosine and its possible applications in medicine. **Advances in clinical and experimental medicine: official organ Wroclaw Medical University**. 22(5):739-744.

Cararo, J. H., Streck, E. L., Schuck, P. F., and da C Ferreira, G. (2015). Carnosine and related peptides: therapeutic potential in age-related disorders. **Aging and disease**. 6(5):369.

Carnegie, P. R., Ilic, M. Z., Etheridge, M. O., and Collins, M. G. (1983). Improved high-performance liquid chromatographic method for analysis of histidine dipeptides anserine, carnosine and balenine present in fresh meat. **Journal of Chromatography A**. 261:153-157.

Caruso, J., Charles, J., Unruh, K., Giebel, R., Learmonth, L., and Potter, W. (2012). Ergogenic effects of β-alanine and carnosine: proposed future research to quantify their efficacy. **Nutrients.** 4(7):585-601.

Castro-Puyana, M., Pérez-Míguez, R., Montero, L., and Herrero, M. (2017). Reprint of: Application of mass spectrometry-based metabolomics approaches for food safety, quality and traceability. **TrAC Trends in Analytical Chemistry**. 96:62-78.

Chan, K., De Cker, E., and Means, W. (1993). Extraction and activity of carnosine, a naturally occurring antioxidant in beef muscle. **Journal Food Science.** 58(1): 1–4.

Cong, J., Zhang, L., Li, J., Wang, S., Gao, F., & Zhou, G. (2017). Effects of dietary supplementation with carnosine on growth performance, meat quality, and antioxidant capacity and muscle fiber characteristics in broiler chickens. **Journal of the Science of Food and Agriculture**. 97(11):3733-3741.

Cornet, M. and J. Bousset. 1999. Free amino acids anddipeptides in porcine muscles: Differences between‘‘red’’ and ‘‘white’’ muscles. **Meat Science**. 51: 215–219.

Culbertson, J. Y., Kreider, R. B., Greenwood, M., and Cooke, M. (2010). Effects of beta-alanine on muscle carnosine and exercise performance: a review of the current literature. **Nutrients.** 2(1):75-98.

Currell, K., Derave, W., Everaert, I., McNaughton, L., Slater, G., Burke, L. M., and Castell, L. M. (2011). A–Z of nutritional supplements: dietary supplements, sports nutrition foods and ergogenic aids for health and performance–Part 20. **British Journal of Sports Medicine.** 45(6):530-532.

Davis, R., and Mauer, L. J. (2010). Fourier transform infrared (FT-IR) spectroscopy: a rapid tool for detection and analysis of foodborne pathogenic bacteria. **Current research, technology and education topics in applied microbiology and microbial biotechnology**. 2:1582-1594.

Decker, E. A., and Mei, L. O. N. G. Y. U. A. N. (1996). Antioxidant mechanisms and applications in muscle foods. **International Journal of Heat and Mass Transfer.** 31:923-930.

Derave, W., Everaert, I., Beeckman, S., and Baguet, A. (2010). Muscle carnosine metabolism and β- alanine supplementation in relation to exercise and training. **Sports medicine.** 40(3): 247-263.

Drozak, J., Chrobok, L., Poleszak, O., Jagielski, A. K., and Derlacz, R. (2013) Molecular identification of carnosine N-methyltransferase as chicken histamine N-methyltransferase-like protein (hnmt-like). **PLoS One.** 8(5): e64805.

Drozak, J., Veiga-da-Cunha, M., Vertommen, D., Stroobant, V., and Van Schaftingen, E. (2010). Molecular identification of carnosine synthase as ATP-grasp domain-containing protein 1 (ATPGD1). **Journal of biological chemistry**. 285(13): 9346-9356.

Everaert, I., De Naeyer, H., Taes, Y., and Derave, W. (2013). Gene expression of carnosine-related enzymes and transporters in skeletal muscle. **European journal of applied physiology.** 113 (5):1169-1179.

Harris, R. C., Wise, J. A., Price, K. A., Kim, H. J., Kim, C. K., and Sale, C. (2012). Determinants of muscle carnosine content. **Amino acids.** 43(1): 5-12.

Hoffman, J. R., Stout, J. R., Harris, R. C., and Moran, D. S. (2015). Β-Alanine supplementation and military performance. **Amino acids**. 47(12):2463-2474.

Holeček, M. (2020). Histidine in health and disease: metabolism, physiological importance, and use as a supplement. **Nutrients.** 12(3):848.

Iruloh, C. G., D'Souza, S. W., Speake, P. F., Crocker, I., Fergusson, W., Baker, P. N., and Glazier, J. D. (2007). Taurine transporter in fetal T lymphocytes and platelets: differential expression and functional activity. **American Journal of Physiology-Cell Physiology.** 292(1): C332-C341.

Jargin, S. V. (2016). On the use of carnosine and antioxidants: A letter from Russia. **Journal of intercultural ethnopharmacology.** 5(3):317.

Jones, G., Smith, M., and Harris, R. (2011). Imidazole dipeptide content of dietary sources commonly consumed within the British diet. **Proceedings of the Nutrition Society.** 70: 363–372.

Jung, S., Bae, Y. S., Kim, H. J., Jayasena, D. D., Lee, J. H., Park, H. B., and Jo, C. (2013). Carnosine, anserine, creatine, and inosine 5′-monophosphate contents in breast and thigh meats from 5 lines of Korean native chicken. **Poultry Science.** 92(12): 3275-3282.

Kai, S., Watanabe, G., Kubota, M., Kadowaki, M., and Fujimura, S. (2015). Effect of dietary histidine on contents of carnosine and anserine in muscles of broilers. **Animal Science Journal.** 86(5): 541-546.

Katemala, S., Molee, A., Thumanu, K., and Yongsawatdigul, J. (2021). Meat quality and Raman spectroscopic characterization of Korat hybrid chicken obtained from various rearing periods. **Poultry Science.** 100(2): 1248-1261.

Khosravi, M., Rahimi, R., and Safavib, E. (2014). Synthesis of L-carnosine and its applications in biomedical fields. **In The 18th International Electronic Conference on Synthetic Organic Chemistry. Multidisciplinary Digital Publishing Institute.** 2014.

Kim, H. C., Yim, D. G., Kim, J. W., Lee, D., and Jo, C. (2021). Nuclear magnetic resonance (NMR)-based quantification on flavor-active and bioactive compounds and application for distinguishment of chicken breeds. **Food Science of Animal Resources**. 41(2):312.

Kim, S. K., Kim, Y. M., Baek, I. K., and Auh, J. H. (2012). Carnosine and anserine in chicken: Distribution, age-dependency and their anti-glycation activity. **Korean Journal for Food Science of Animal Resources.** 32(1): 45-48.

Kojima, S., Saegusa, H., and Sakata, M. (2014). Histidine-containing dipeptide concentration and antioxidant effects of meat extracts from silky fowl: comparison with meat-type chicken breast and thigh meats. **Food Science and Technology Research.** 20(3): 621-628.

Kralik, G., Sak-Bosnar, M., Grčević, M., and Kralik, Z. (2018). Effect of Amino Acids on Growth Performance, Carcass Characteristics, Meat Quality, and Carnosine Concentration in Broiler Chickens. **The Journal of Poultry Science**. 0170083.

Kralik, G., Sak-Bosnar, M., Kralik, Z., Galović, O., Grčević, M., and Kralik, I. (2015). Effect of β-alanine and L-histidine on concentration of carnosine in muscle tissue and oxidative stability of chicken meat. **PoljoPrivreda.** 21(1 SUPPLEMENT): 190-194

Kukurba, K. R., & Montgomery, S. B. (2015). RNA sequencing and analysis. **Cold Spring Harbor Protocols**. 2015(11):pdb-top084970.

Lee, H.J., Jayasena, D.D., Kim, S.H., Kim, H.J., Heo, K.N., Song, J.E., and Jo, C. (2015). Comparison of bioactive compounds and quality traits of breast meat from Korean native ducks and commercial ducks. **Korean Journal for Food Science of Animal Resources.** 35(1): 114–120.

Lenney, J. F., Peppers, S. C., Kucera-Orallo, C. M., and George, R. P. (1985). Characterization of human tissue carnosinase. **Biochemical Journal**. 228(3): 653-660.

Li, S., Tian, Y., Jiang, P., Lin, Y., Liu, X., & Yang, H. (2021). Recent advances in the application of metabolomics for food safety control and food quality analyses. **Critical Reviews in Food Science and Nutrition**. 61(9):448-1469.

Liu, L., Xiao, Q., Gilbert, E. R., Cui, Z., Zhao, X., Wang, Y., and Zhu, Q. (2018). Whole-transcriptome analysis of atrophic ovaries in broody chickens reveals regulatory pathways associated with proliferation and apoptosis. **Scientific reports.** 8(1):7231.

Ma, X. Y., Jiang, Z. Y., Lin, Y. C., Zheng, C. T., and Zhou, G. L. (2010). Dietary supplementation with carnosine improves antioxidant capacity and meat quality of finishing pigs. **Journal of Animal Physiology and Animal Nutrition**. 94: e286–e295.

Maikhunthod, B. and K. O. Intarapichet. (2005). Heat and ultrafiltration extraction of broiler meat carnosine and its antioxidant activity. **Meat Science**. 71:364-374.

McDonough, C. W., Hicks, P. J., Lu, L., Langefeld, C. D., Freedman, B. I., and Bowden, D. W. (2009). The influence of carnosinase gene polymorphisms on diabetic nephropathy risk in African-Americans. **Human genetics**. 126(2):265-275.

Milner, J. A. (1999). Functional foods and health promotion. **The Journal of nutrition**. 129(7):1395S-1397S.

Miyaji, T., Sato, M., Maemura, H., Takahata, Y., and Morimatsu, F. (2012). Expression profiles of carnosine synthesis–related genes in mice after ingestion of carnosine or β-alanine. **Journal of the International Society of Sports Nutrition.** 9(1): 15.

Mong, M. C., Chao, C. Y., & Yin, M. C. (2011). Histidine and carnosine alleviated hepatic steatosis in mice consumed high saturated fat diet. **European journal of pharmacology**. 653(1-3):82-88.

Mora, L., Sentandreu, M.Á., and Toldrá, F. (2008). Contents of creatine, creatinine and carnosine in porcine muscles of different metabolic types. **Meat Science**. 79(4): 709–715.

Mori, M., Mizuno, D., Konoha-Mizuno, K., Sadakane, Y., and Kawahara, M. (2015). Quantitative analysis of carnosine and anserine in foods by performing high performance liquid chromatography. **Biomedical Research on Trace Elements**. 26(3):147-152.

Muthubharathi, B. C., Gowripriya, T., and Balamurugan, K. (2021). Metabolomics: small molecules that matter more. **Molecular Omics**. 17(2):210-229.

Nisa, A. U., Zahra, N., Sajila, H., Masood, S., Javed, A., and Manzarinam, S. (2017). Development of meat-based functional foods: A review. **Journal of Applied Biology & Biotechnology.** 5(3):086-092.

Peiretti, P. G., Medana, C., Visentin, S., Dal Bello, F., and Meineri, G. (2012). Effect of cooking method on carnosine and its homologues, pentosidine and thiobarbituric acid-reactive substance contents in beef and turkey meat. **Food Chemistry.** 132(1):80-85.

Perim, P., Marticorena, F. M., Ribeiro, F., Barreto, G., Gobbi, N., Kerksick, C. M., and Saunders, B. (2019). Can the skeletal muscle carnosine response to beta-alanine supplementation be optimised?. **Frontiers in Nutrition**. 6:135.

Pfister, F., Riedl, E., Wang, Q., vom Hagen, F., Deinzer, M., Harmsen, M. C., and Hammes, H. P. (2011). Oral carnosine supplementation prevents vascular damage in experimental diabetic retinopathy. **Cellular Physiology and Biochemistry**. 28(1):125-136.

Poompramun, C., Molee, W., Thumanu, K., and Molee, A. (2021). The significant influence of Residual Feed Intake (RFI) on flavor precursors and

biomolecules in slow-growing Korat chicken meat. **Animal Bioscience**. 34(10):1684.

Pramote, P. (2016). Situation of the Thai elderly 2015. **Bangkok: Amarin printing & publishing public Co., Ltd.** 2016.

Purchas, R.W., Rutherfurd, S.M., Pearce, P.D., Vather, R., and Wilkinson, B.H. (2004). Cooking temperature effects on the forms of iron and levels of several other compounds in beef semitendinosus muscle. **Meat Science**. 68(2): 201–207.

Qi, B., Wang, J., Ma, Y. B., Wu, S. G., Qi, G. H., and Zhang, H. J. (2018). Effect of dietary β-alanine supplementation on growth performance, meat quality, carnosine content, and gene expression of carnosine-related enzymes in broilers. **Poultry Science**. 97(4):1220-1228.

Rajasekaran, A., and Kalaivani, M. (2013). Designer foods and their benefits: A review. **Journal of food science and technology**. 50(1):1-16.

Rankin, N. J., Preiss, D., Welsh, P., Burgess, K. E., Nelson, S. M., Lawlor, D. A., and Sattar, N. (2014). The emergence of proton nuclear magnetic resonance metabolomics in the cardiovascular arena as viewed from a clinical perspective. **Atherosclerosis**. 237(1):287-300.

Riedl, E., Pfister, F., Braunagel, M., Brinkkötter, P., Sternik, P., Deinzer, M., and Navis, G. (2011). Carnosine prevents apoptosis of glomerular cells and podocyte loss in STZ diabetic rats. **Cellular physiology and biochemistry.** 28(2):279-288.

Sabino, M., Carmelo, V. A. O., Mazzoni, G., Cappelli, K., Capomaccio, S., Ajmone-Marsan, P. and Kadarmideen, H. N. (2018). Gene co-expression networks in liver and muscle transcriptome reveal sex-specific gene expression in lambs fed with a mix of essential oils. **BMC genomics**. 19(1):236.

Sadikali, F., Darwish, R., and Watson, W. C. (1975). Carnosinase activity of human gastrointestinal mucosa. **Gut**. 16(8):585-589.

Sale, C., Saunders, B., and Harris, R. C. (2010). Effect of beta-alanine supplementation on muscle carnosine concentrations and exercise performance. **Amino acids**. 39(2):321-333.

Saputra, I., Jaswir, I., and Akmeliawati, R. (2018). Profiling of Wavelength Biomarkers of Pure Meat Samples from Different Species Based on Fourier Transform Infrared Spectroscopy (FTIR) and PCA Techniques. **International Journal on**

Advanced Science, Engineering and Information Technology. 8(4-2): 1617-1624.

Simmler, C., Napolitano, J. G., McAlpine, J. B., Chen, S. N., and Pauli, G. F. (2014). Universal quantitative NMR analysis of complex natural samples. **Current opinion in biotechnology.** 25:51-59.

Sinpru, P., Riou, C., Kubota, S., Poompramun, C., Molee, W., and A. Molee. 2021. Jejunal transcriptomic profiling for differences in feed conversion ratio in slow-growing chickens. **Animals**. 11(9):2606.

Son, D.O., Satsu, H., Kiso, Y., and Shimizu, M. (2004). Characterization of carnosine uptake and substance contents in beef and turkey meat. **Food Chemistry.** 132(1): 80–85.

Suwanvichanee, C., Sinpru, P., Promkhun, K., Kubota, S., Riou, C., Molee, W., and Molee, A. (2022). Effects of β-alanine and L-histidine supplementation on carnosine contents in and quality and secondary structure of proteins in slow-growing Korat chicken meat. **Poultry Science**. 101(5):101776.

Tanaka, S., & Ichikawa, A. (2006). Recent advances in molecular pharmacology of the histamine systems: immune regulatory roles of histamine produced by leukocytes. **Journal of Pharmacological Sciences.** 0604270009-0604270009.

Teufel, M., Saudek, V., Ledig, J. P., Bernhardt, A., Boularand, S., Carreau, A., and Smirnova, T. (2003). Sequence identification and characterization of human carnosinase and a closely related non-specific dipeptidase. **Journal of Biological Chemistry**. 278(8):6521-6531.

Thumanu, K., Sangrajrang, S., Khuhaprema, T., Kalalak, A., Tanthanuch, W., Pongpiachan, S., and Heraud, P. (2014). Diagnosis of liver cancer from blood sera using FTIR microspectroscopy: a preliminary study. **Journal of biophotonics.** 7(3-4):222-231.

Tian, Y., M. Xie, W. Wang, H. Wu, Z. Fu, and L. Lin. (2007). Determination of carnosine in Black-Bone Silky Fowl (Gallus gallus domesticus Brisson) and common chicken by HPLC. **European Food Research and Technology.** 226:311-314.

Tomonaga, S., Matsumoto, M., and Furuse, M. (2012). Β-Alanine enhances brain and muscle carnosine levels in broiler chicks. **The Journal of Poultry Science**. 49(4): 308-312.

Varanoske, A. N., Stout, J. R., and Hoffman, J. R. (2019). Effects of β-Alanine Supplementation and Intramuscular Carnosine Content on Exercise Performance and Health. **In Nutrition and Enhanced Sports Performance** (pp. 327-344). Academic Press.

Wan, Y., Jin, S., Ma, C., Wang, Z., Fang, Q., and Jiang, R. (2017). RNA-Seq reveals seven promising candidate genes affecting the proportion of thick egg albumen in layer-type chickens. **Scientific reports.** 7(1):18083.

Wang, J. P., Yang, Z. T., Liu, C., He, Y. H., and Zhao, S. S. (2013). L-carnosine inhibits neuronal β-alanine transamination. **The Journal of Physiology**. 594(17): 4849-4863.

Wang, Z., Gerstein, M., and Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. **Nature Reviews Genetics**. 10(1):57.

Wen, D., Liu, Y., and Yu, Q. (2020). Metabolomic approach to measuring quality of chilled chicken meat during storage. **Poultry Science**. 99(5):2543-2554.

Wishart, D. S. (2013). Exploring the human metabolome by nuclear magnetic resonance spectroscopy and mass spectrometry. **Methodologies for Metabolomics: Experimental Strategies and Techniques; Lutz, NW, Sweedler, JV, Wevers, RA, Eds**. 3-29.

Xing, L., Chee, M. E., Zhang, H., Zhang, W., and Mine, Y. (2019). Carnosine-a natural bioactive dipeptide: bioaccessibility, bioavailability and health benefits. **Journal of Food Bioactives**. 5:8-17.

Yang, P., Hao, Y., Feng, J., Lin, H., Feng, Y., Wu, X., and Gu, X. (2014). The expression of carnosine and its effect on the antioxidant capacity of longissimus dorsi muscle in finishing pigs exposed to constant heat stress. **Asian-Australasian journal of animal sciences**. 27(12): 1763.

Zanella, P. B., Alves, F. D., and de Souza, C. G. (2016). Effects of beta-alanine supplementation on performance and muscle fatigue in athletes and non-

athletes of different sports: a systematic review. **Clinical and Experimental Pediatrics.** 90035(003).

Zhang, J., Nie, Q., Si, C., Wang, C., Chen, Y., Sun, W. and Wang, F. (2019). Weighted gene co-expression network analysis for RNA-Sequencing data of the varicose veins transcriptome. **Frontiers in physiology**. 10:278.

Zhang, T., Chen, C., Xie, K., Wang, J., and Pan, Z. (2021). Current state of metabolomics research in meat quality analysis and authentication. **Foods.** 10(10):2388.

Zhu, C., Petracci, M., Li, C., Fiore, E., and Laghi, L. (2020). An untargeted metabolomics investigation of Jiulong yak (Bos grunniens) meat by 1H-NMR. **Foods**. 9(4):48.

CHAPTER III

EFFECT OF CARNOSINE SYNTHESIS PRECURSORS IN THE DIET ON JEJUNAL METABOLOMIC PROFILING AND BIOCHEMICAL COMPOUNDS IN SLOW-GROWING KORAT CHICKEN

3.1 Abstract

The slow-growing Korat chicken (**KR**) has been developed to provide an alternative breed for smallholder farmers in Thailand. Carnosine enrichment in the meat can distinguish KR from other chicken breeds. Therefore, we aimed to investigate the effect of enriched carnosine synthesis, obtained by the β-alanine and L-histidine precursor supplementation in the diet, on changes to metabolomic profiles and biochemical compounds in slow-growing KR jejunum tissue. Four hundred 21-day-old female KR chickens were divided into four experimental groups: a group with a basal diet, a group with a basal diet supplemented with 1.0% β-alanine, 0.5% L-histidine, and a mix of 1.0% β-alanine and 0.5% L-histidine. The feeding trial lasted 70 days. Ten randomly selected chickens from each group were slaughtered. Metabolic profiles were analyzed using proton nuclear magnetic resonance spectroscopy. In total, 28 metabolites were identified. Significant changes in the concentrations of these metabolites were detected between the groups. Partial least squares discriminant analysis was used to distinguish the metabolites between the experimental groups. Based on the discovered metabolites, 34 potential metabolic pathways showed differentiation between groups, and eight pathways (with impact values higher than 0.05, $P < 0.05$, and FDR < 0.05) were affected by metabolite content. In addition, biochemical changes were monitored using synchrotron radiation-based Fourier transform infrared microspectroscopy. Supplementation of β-alanine alone in the diet increased the β-sheets and decreased the α-helix content in the amide I region, and supplementation of L-histidine alone in the diet also increased the β-sheets. Furthermore, the relationship between metabolite contents and biochemical

compounds were confirmed using principal component analysis (**PCA**). Results from the PCA indicated that group D was highly positively correlated with amide I, amide II, creatine, tyrosine, valine, isoleucine, and aspartate. These findings can help to understand the relationships and patterns between the spectral and metabolic processes related to carnosine synthesis.

Keywords: β-alanine, L-histidine, carnosine synthesis, jejunum, metabolite

3.2 Introduction

The Korat chicken (**KR**) is a crossbreed between the Thai indigenous chicken Leung Hang Khao (sires) and the Suranaree University of Technology synthetic breed (dams). KR was developed to provide an alternative breed for the smallholder farmers in Thailand. The main disadvantage of KR is their relatively slow growth rate, and female KR has low performance compared with other commercial breeds (Poompramun et al., 2021). The main advantages of KR are their higher carnosine content, improved water retention ability, and pH_{45} of the meat (Suwanvichanee et al., 2022). Consuming meat with a high content of carnosine has a favorable effect on human health, such as the prevention of diverse age-related diseases (Hipkiss et al., 2013). Besides improving the growth rate of KR, its competitiveness in the Thai chicken industry can be further improved by increasing the carnosine content in KR meat, given that, in the future, consumers may prefer foods that have favorable effects on health.

Carnosine is a cytoplasmic dipeptide synthesized by the bonding of amino acids β-alanine and L-histidine (Perim et al., 2019; Xing et al., 2019; Jukić et al., 2021). It is found at particularly high concentrations in chicken muscles (Jukić et al., 2021). β-alanine can be obtained through the hepatic breakdown of thymidine, uracil, and dietary dipeptides and is considered a non-proteinogenic rate-limiting precursor of carnosine (Derave et al., 2014). L-histidine is an essential amino acid present in serum and serves as a proteinogenic precursor with bioactive properties (Xing et al., 2019). Catalyzed by the carnosinase enzyme, carnosine degradation occurs in the serum and tissues through hydrolysis before being transmitted to blood vessels by amino transporters (Sale et al., 2013; Harris et al., 2012), and both amino acids can

subsequently reassemble as carnosine in the tissue through carnosine synthetase (Blancquaert et al., 2017). In a previous study, β-alanine and L-histidine supplementation in diets enhances carnosine synthesis in the breast muscle without any adverse effect on growth performance, meat quality, and meat texture (Suwanvichanee et al., 2022). Moreover, dietary supplementation with L-histidine alone softens meat toughness in KR chickens (Kubota et al., 2021).

Animals extensively catabolize amino acids in their small intestine. Based on human and mice studies, the jejunum has the highest ability to absorb carnosine compared with other parts of the small intestine because of the high carnosinase enzyme activity of the enterocytes in the jejunal mucosa that act against the carnosine substrate (Sadikali et al., 1975; Ferraris et al., 1988; Sale et al., 2013). However, the metabolic profiling associated with the carnosine synthesis pathway in chickens has not been studied previously. Therefore, in this study, we used untargeted metabolomics and Fourier transform infrared (**SR-FTIR**) microspectroscopy to investigate the metabolic and biochemical changes in the jejunum of KR chickens fed with or without a combination of β-alanine and L-histidine, precursors for carnosine synthesis differentiation, in the diet.

Given that β-alanine and L-histidine are the precursors of carnosine synthesis, our research question is whether different dietary supplementations of the carnosine precursors will provide different mechanisms and/or pathways for the absorption and transportation of the amino acids in the jejunum for carnosine synthesis in meat. Therefore, our main objective is to investigate biochemical compounds, metabolic profiles, and metabolic pathways in the jejunum related to carnosine accumulation in the meat that can be used in the KR chicken breeding program to improve competitiveness in the Thai chicken meat industry.

3.3 Materials and methods

3.3.1 Ethics Statement

All animal protocols used in this research were approved by the Ethics Committee on Animal Use of the Suranaree University of Technology, Nakhon Ratchasima, Thailand; document ID U1-02631-2559.

3.3.2 Experimental Design and Sample Collection

We used 40 female KR chickens in this study. Details of the experiment are given in a previous study by Suwanvichanee et al. (2022). Briefly, the experiment was conducted at the experimental farm of the Suranaree University of Technology (SUT), Thailand using a total of 400 female KR chickens. Feeding included three phases based on chicken age: a starter diet for chickens 1 to 21 days of age (21% protein in diet), a growing phase for chickens 22 to 42 days of age (19% protein in diet), and a finisher diet for chickens 43 to 70 days of age (17% protein in diet). For more details on the composition of experimental feed, see Suwanvichanee et al. (2022). At the age of 28 days, the chickens were divided into four experimental groups: a group with a basal diet, a group with a basal diet supplemented with 1.0% (1 g/100 g diet) β-alanine, a group with a basal diet supplemented with 0.5% (0.5 g/100 g diet) L-histidine, and a group with a basal diet supplemented with 1.0% of β-alanine and 0.5% L-histidine. Each experimental group was divided into five replicates with 20 chickens. Feed and water were supplied automatically on ad libitum basis. A vaccination program was conducted under recommendation of the Department of Livestock Development, Bangkok, Thailand. The growth performance of chickens, i.e. feed intake, body weight gain, average daily feed intake, average daily gain, body weight, and feed conversion ratio, were recorded weekly.

At 70 days of age, 10 randomly selected chickens from each experimental group (two chickens per replicate) were tagged, stunned by chloroform, exsanguinated by cutting the jugular vein, and allowed to bleed for approximately 2 min. We collected five to ten pieces of 2-cm long whole jejunum segments. For SR-FTIR microspectroscopy measurements, the whole jejunum was cut into 3–5-mm thick pieces, washed in a normal saline solution, and stored in 10% buffered formaldehyde. Afterward, the samples were refrigerated for 24 h and transferred to a medium containing 80% ethanol until they were embedded in paraffin wax. In addition, a small amount of the whole jejunum was collected, immediately frozen in liquid nitrogen, and stored at −80°C for metabolomic analysis.

3.3.3 Prepared proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) Analyses

Metabolite extraction was performed according to Le Roy et al. (2016). The 40 frozen jejunum samples were thawed at 4°C for 1 hour. Then, 0.1 g of tissues were mixed with 1 mL of a 3:1 (v/v) MeOH/H_2O solution, homogenized for 5 min in a bead beater (BioSpec Products, Inc., Mini-Beadbeater-1 6, United States), and centrifuged at 12,000 × g for 10 min at 4°C. The 250 µL of the supernatant extracts were transferred into a 1.5-mL tube, dried in a glass vacuum desiccator for 5–6 hours at room temperature, and stored at −20°C.

Before ^{1}H-NMR analysis, the samples were resuspended in 250 µL of deionized water and 250 µL of 99.9 % Deuterium oxide (D_2O) (MagniSolv™, Darmstadt, Germany). Then, the samples were mixed and 500 uL were transferred into a 5-mm NMR tube (Norwell, USA) for the ^{1}H-NMR analysis. Spectra were collected using a 500-MHz NMR spectrometer (Bruker AVANCE III HD, Germany) equipped with a CryoProbe-CPP BBO 500S1. The first increment of a 2D-1H, 1H- nuclear-overhauled effect spectroscopy (NOESY) pulse sequence was utilized to acquire ^{1}H-NMR data and suppress the solvent signal. Each spectrum consisted of 32,768 data points collected at 25°C with 128 scans over 16.0 min.

3.3.4 Metabolite Identification, Quantification, and Pathway Analysis

Metabolites were determined manually based on chemical shifts (Figure 3.1, see the labels of the metabolites from Table 3.1). All points of the free induction decays (FID) were Fourier-transformed, and missing values were filled with TOPSPIN software (Version 3.0, Bruker Biospin, Germany). The baseline was corrected to the reference resonance at δ = 0.0 ppm with MestReNova 14.2.0-26256 software (Mestrelab Research S.L., Santiago de Compostela, Spain). Spectral regions related to residual water (4.70-5.10 ppm) were removed (Le Roy et al., 2016) to avoid the effects of imperfect water suppression. In the first step of the process, the 0.5-8.5-ppm chemical shift (δ) region was integrated into regions with a width of 0.04 ppm. The data were normalized by the sum over all integral regions using the ACD/NMR predictor. The Chenomx Compound Library was used to compare all the spectra to the internal standard DSS (Xiao et al., 2019).

Statistical analyses of the modified spectra data of various experimental groups was conducted using the MetaboAnalyst 5.0 platform (http://www.metaboanalyst.ca/). Before statistical analyses, the data were log-transformed and Pareto-scaled (mean-centered and divided by the standard deviation) (Abreu and Fernández, 2020). Data dimensionality was reduced using the supervised partial least squares discriminant analysis (**PLS-DA**), and leave-one-out cross-validation was used for measuring performance accuracy. The coefficient of determination (R^2) and predictive ability (Q^2) were used as initial indicators of the goodness of the model fit. Significance of the metabolites at discriminating the samples was based on the variable importance in the projection (**VIP**) measurement (Xiao et al., 2019); a high VIP value indicates a high contribution of the corresponding metabolite in the discrimination of samples. VIP values greater than 1 were considered significant. For each significant variable, differences between the treatment groups were tested with analysis of variance (one-way ANOVA) using SPSS (SPSS for Windows, version 16.0, SPSS Inc., Chicago, Illinois). The pair-wise differences between the treatments were tested with the Tukey's HSD post hoc test. Differences with a $P < 0.05$ were considered statistically significant.

The pathway analyses were conducted using the Gallus gallus library in the MetaboAnalyst 5.0 platform. Given the exploratory nature of the study, we considered pathways with a $P < 0.05$ to be highly interesting.

3.3.5 SR-FTIR Microspectroscopy

Paraffin-embedded tissue was washed in 70% ethanol to remove the fixing solutions, dehydrated in graded series of alcohol, cleared in xylol, and embedded into paraffin blocks. Sections were cut with a rotary microtome (HM 340E Electronic, Thermo Fisher, Scientific Microm International GmbH, Germany) into 10-μm thick pieces, mounted onto a barium fluoride (BaF_2) (Crystran Ltd., Dorset, UK) window for spectral acquisition, and dried in a vacuum desiccator before measurement.

The spectra were measured using a Bruker FTIR spectrometer (Vertex70, Bruker Optics, Ettlingen, Germany) coupled with a Bruker Hyperion 2000-IR Microscopy (Bruker Optik GmbH, Ettlingen, Germany) using the 36x IR objective lens with a mercury cadmium telluride (MCT) detector cooled with liquid nitrogen over the measurement range from 4000 to 800 cm^{-1}. The transmission mode was used to obtain 64 scans with

a 10 × 10 μm aperture size at a resolution of 6 cm^{-1}. Spectral acquisition and instrument control were performed using OPUS 7.5 software (Bruker Optics Ltd, Ettlingen, Germany). The background was collected through a blank substrate before spectral analysis. FTIR spectra were measured using synchrotron-based FTIR (Fourier Transform Infrared) spectroscopy (BL4.1 Infrared Spectroscopy and Imaging, Synchrotron Light Research Institute, Nakhon Ratchasima, Thailand).

The spectra data were processed with OPUS 7.5 software (Bruker Optics Ltd.). The percentage of integral areas was determined using second-derivative processing from 1700 to 1600 cm^{-1} (amide I); 1600 to 1500 cm^{-1} (amide II); 1450 to 1390 cm^{-1} (CH bending); 1320 to 1220 cm^{-1} (amide III); and 1200 to 900 cm^{-1} (glycogen and carbohydrate). For the overlapping peaks of the amide I band, the curve fitting based on the Gaussian and Lorentzian functions was applied. The fitting parameters were determined in several regions: β-sheet (1630 cm^{-1}), α-helix (1644, 1655 cm^{-1}), β-turn (1670 cm^{-1}), and antiparallel (1689 cm^{-1}).

3.3.6 Principal Component Analysis (PCA) of SR-FTIR Spectral Data and Metabolomics

The PCA was applied to cluster and investigate the relationships between the metabolite parameters (with VIP score > 1), the biochemical compounds (amide I, amide II, CH-bending, amide III, and glycogen/carbohydrate), and the secondary structure ratios (α-helix, β-sheet, β-turn, and antiparallel) using the Unscrambler® X Multivariate Data Analysis software (version 10.1, Camo Analytics, Oslo, Norway). The correlations between the variables were represented by a correlation loading plot. Before forming the correlation loading plot, the variables were weighted based on the standard deviation of the variable.

3.4 Results and Discussion

3.4.1 Growth Performance Parameter

No significant difference in growth performance was found between the treatment groups. The data on growth performance is published in Suwanvichanee et al. (2022) shown in Table 3.1.

Table 3.1 Effect of amino acids supplementation on growth performance and carnosine and anserine contents in KR breast meat. (Source: Suwanvichanee et al., 2022).

Parameter	Treatment group[1]				SEM[2]	P-value
	A	B	C	D		
22-42 d						
FI (g)	1018.64	1092.50	1037.32	1038.99	18.67	0.07
BWG (g)	381.97	386.21	382.48	390.17	5.68	0.72
ADFI (g)	48.51	52.02	49.40	49.47	0.89	0.07
ADG (g)	18.19	18.39	18.21	18.58	0.27	0.72
BW 42 d (g)	635.97	643.21	637.98	644.17	6.10	0.74
FCR	2.67	2.83	2.71	2.66	0.05	0.15
43-70 d						
FI (g)	1960.78	1940.13	1964.78	1933.38	59.71	0.98
BWG (g)	512.64	501.04	523.21	562.64	24.05	0.33
ADFI (g)	70.03	69.29	70.17	69.05	2.13	0.98
ADG (g)	18.31	17.90	18.69	20.09	0.89	0.33
BW 70 d (g)	1148.61	1144.25	1161.19	1206.81	26.01	0.34
FCR	3.85	3.91	3.77	3.46	0.19	0.38
22-70 d						
FI (g)	2979.42	3032.63	3002.10	2972.37	68.99	0.93
BWG (g)	894.61	887.25	910.69	952.81	25.65	0.30
ADFI (g)	60.80	61.89	61.27	60.66	1.41	0.93
ADG (g)	18.26	18.11	18.48	19.45	0.52	0.30
FCR	3.34	3.43	3.32	3.13	0.12	0.36
Carnosine (µg/g)	2,756.6^{c}	3,484.6^{b}	3,659.8^{b}	4,212.5^{a}	82.88	<0.001
Anserine (µg/g)	10,577.2	10,391.6	10,312.7	10,272.8	282.47	0.88

1Treatment groups are A (control), B (supplemented with 1.0% β-alanine), C (supplemented with 0.5% L-histidine), and D (supplemented with 1.0% β-alanine + 0.5% L-histidine) respectively.

2SEM indicates standard error of mean (mean ± SEM; n = 5/treatment).

FI, feed intake; BWG, body weight gain; ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; FCR, feed conversion ratio.

3.4.2 Metabolite Profile Analysis

Based on the ^{1}H NMR spectra, 28 metabolites were unambiguously identified in the jejunum (Figure 3.1).

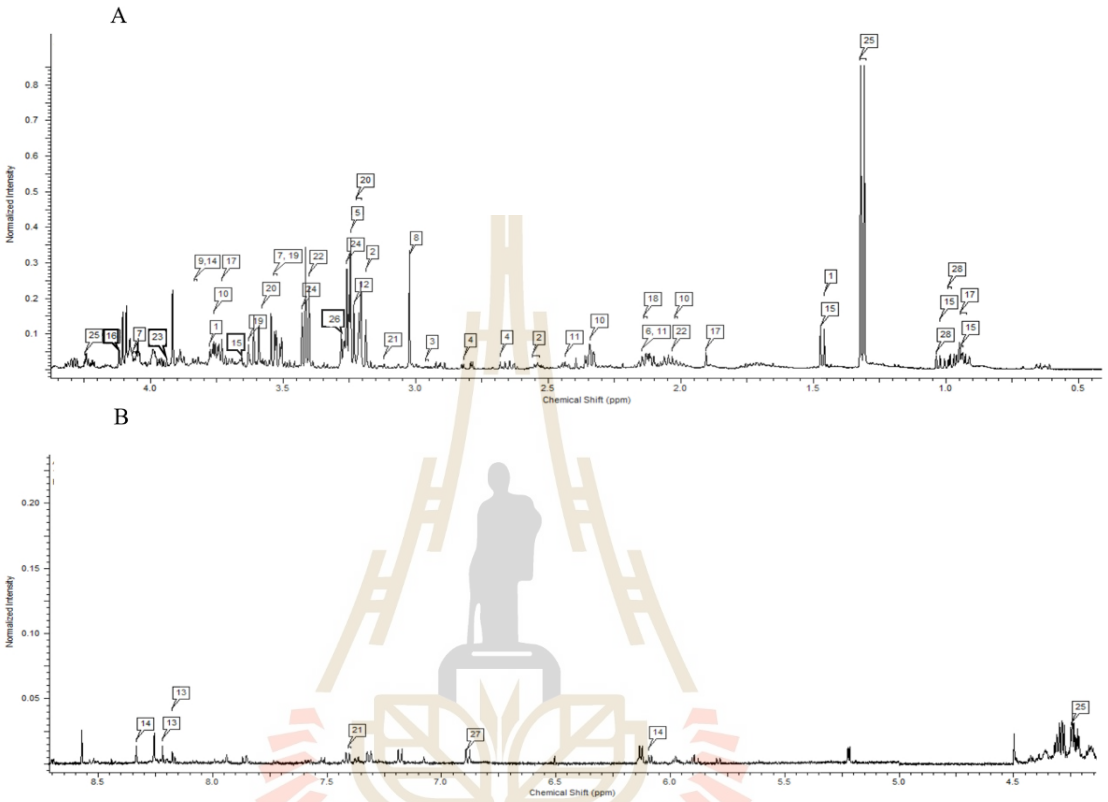

Figure 3.1 Representation of ^{1}H-NMR spectrum at 0.5–4.1 ppm chemical shift (δ) region (A) and 4.4–8.5 ppm chemical shift (δ) region (B).

In most cases, the groups fed with carnosine synthesis precursors had higher metabolite levels in the jejunum tissue compared to the control group (Table 3.2). Based on a previous study by Suwanvichanee et al. (2022), supplementation of both β-alanine and L-histidine, i.e., precursors of carnosine synthesis, in the diet (the same diet as in group D) increases carnosine content in the breast meat. In our study, higher concentrations of β-alanine, butyrate, choline, creatine, glutamate, glutamine, lactate, methionine, and myo-inositol but lower concentrations of betaine, threonine, and taurine were obtained in the β-alanine and L-histidine precursor group compared to the control group.

Table 3.2 Metabolite contents (ppm) in jejunal tissues of the identified metabolites with assigned chemical shifts in the four experimental groups.

No.	Metabolites	Chemical shifts (ppm), Multiplicity	Experimental groups[1]				*P*-value
			A	B	C	D	
1	Alanine	1.46 d, 3.78 q	1417.44^{b}	1690.90^{a}	1334.72^{b}	1497.20^{b}	< 0.001
2	β-alanine	2.56 t, 3.19 t	1881.58^{c}	2284.48^{b}	2378.68^{b}	2614.92^{a}	< 0.001
3	Asparagine	2.96 dd	48.20^{bc}	97.30^{a}	83.80^{ab}	24.86^{c}	0.016
4	Aspartate	2.68 dd, 2.82 dd	377.50^{a}	391.92^{a}	323.44^{b}	336.98^{b}	< 0.001
5	Betaine	3.25 s	8121.36^{a}	6554.44^{b}	7047.00^{b}	6838.52^{b}	< 0.001
6	Butyrate	2.15 t	417.22^{c}	568.84^{a}	470.24^{b}	542.30^{a}	< 0.001
7	Choline	3.53 dd, 4.06 t	3188.84^{b}	3730.46^{a}	3725.10^{a}	4004.08^{a}	< 0.001
8	Creatine	3.02 s, 3.94 s	257.48^{c}	314.18^{ab}	290.28^{b}	342.08^{a}	< 0.001
9	Ethanolamine	3.13 t, 3.83 t	116.28^{c}	140.16^{a}	108.68^{c}	128.14^{b}	0.001
10	Glutamate	2.02 m, 2.34 m, 3.76 dd	300.94^{b}	329.00^{a}	275.30^{c}	328.34^{a}	0.009
11	Glutamine	2.15 m, 2.44 m	223.62^{b}	271.08^{a}	251.62^{a}	269.90^{a}	< 0.001
12	Histidine	3.23 dd, 7.09 s	2727.18^{b}	2629.32^{bc}	3290.86^{a}	2441.92^{c}	< 0.001
13	Hypoxanthine	8.18 s, 8.21 s	187.1	186.04	182.76	192.18	0.591
14	Inosine	3.83 dd, 4.27 dd, 4.43 dd, 6.09 d, 8.34 s	64.70^{ab}	73.74^{a}	58.08^{ab}	50.54^{b}	0.040
15	Isoleucine	0.94 t, 1.02 d, 1.47 dd, 3.65 d	467.24^{c}	661.36^{a}	561.28^{b}	408.68^{d}	< 0.001
16	Lactate	4.12 q	718.26^{b}	908.26^{a}	893.36^{a}	916.56^{a}	< 0.001
17	Leucine	0.93 d, 0.94 d, 3.73 m, 1.91 m	1181.88^{ab}	1231.94^{a}	1104.46^{b}	1238.98^{a}	< 0.001
18	Methionine	2.13 s, 2.14 m	786.44^{b}	1083.62^{a}	920.88^{ab}	1031.26^{a}	0.013
19	Myo-inositol	3.53 dd, 3.63 t	1035.82^{b}	1235.10^{a}	1232.32^{a}	1302.42^{a}	< 0.001
20	Phosphatidylcholine	3.21 s, 3.58 m	357.64^{c}	469.58^{ab}	480.66^{a}	375.38^{bc}	0.013
21	Phenylalanine	3.12 dd, 3.26 dd, 7.40 m	65.26	81.38	106.46	81.6	0.299
22	Proline	2.03 m, 3.41 m	503.64^{bc}	547.74^{a}	462.62^{c}	544.54^{ab}	< 0.001
23	Serine	3.95 dd, 3.95 dd	227.30^{ab}	253.80^{a}	197.26^{b}	201.12^{b}	< 0.001
24	Taurine	3.43 t	3015.50^{a}	2297.58^{c}	2601.20^{b}	2420.16^{bc}	< 0.001
25	Threonine	1.32 d, 4.25 m	321.38^{a}	253.50^{b}	279.58^{b}	271.48^{b}	< 0.001
26	Trimethylamine N-oxide	3.27 s	1217.78	1086.78	1159.7	1189.02	0.730
27	Tyrosine	3.94 dd, 6.89 m	149.38^{b}	195.04^{a}	122.52^{c}	136.86^{b}	< 0.001
28	Valine	0.98 d, 1.04 d	573.98^{b}	681.08^{a}	480.40^{c}	421.92^{d}	< 0.001

[1]Diet groups. A: control, B: supplementation with 1.0% β-alanine, C: supplementation with 0.5% L-histidine, D: supplementation with 1.0% β-alanine and 0.5% L-histidine
s: singlet, d: doublet, t: triplet, q: quartet, dd: doublet of doublets, and m: multiplet
a–c Significantly different treatment groups with *P*-value < 0.05.

The experimental groups were clearly separated also based on the PLS-DA score plot (Figure 3.2A). The first two components explained 56.6% of the total variance; component 1 accounted for 26.9% and component 2 accounted for 29.7% of the total variance (Figure 3.2A). The R^2 = 0.9994 and Q^2 = 0.9933 values demonstrated reasonable qualities for PLS-DA. The results indicate that the metabolite profile of the control group shares similarities with the profile of the β-alanine precursor group, whereas the differences in metabolite profiles between the other groups are noticeable. The most important metabolites for discriminating the treatment groups (VIP > 1) were β-alanine, choline, myo-inositol, creatine, lactate, aspartate, tyrosine, isoleucine, valine, and taurine (Figure 3.2B).

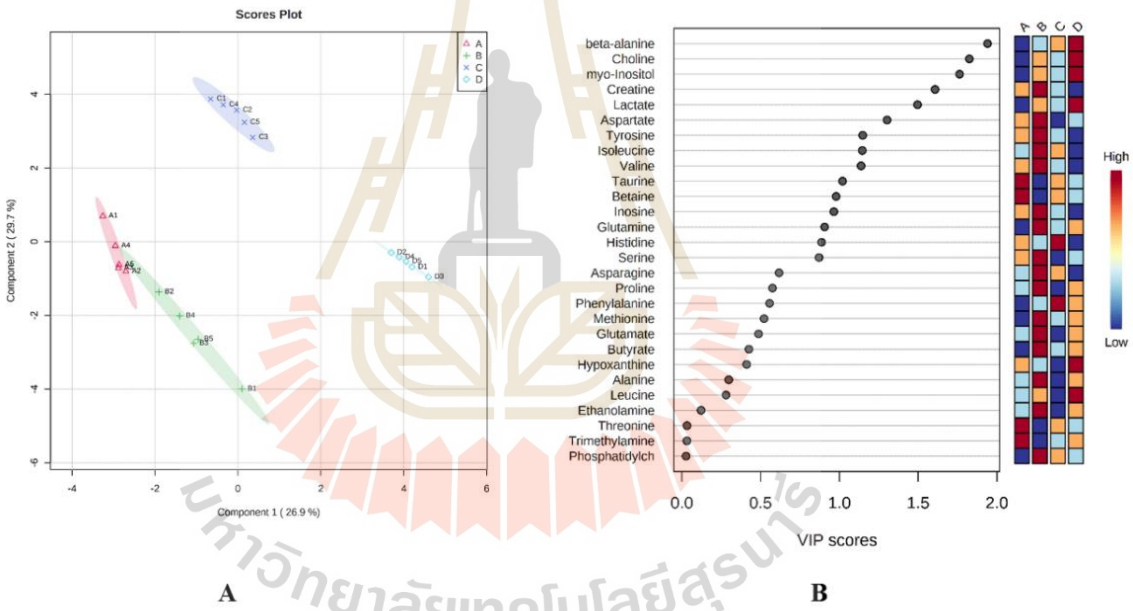

Figure 3.2 Partial least squares discriminant analysis (PLS-DA) score plot (A) and variable importance in projection (VIP) scores (B) based on jejunum metabolites in the four experimental groups; A (control diet), B (diet with 1.0% β-alanine supplementation), C (diet with 0.5% L-histidine supplementation), and D (diet with 1.0% β-alanine and 0.5% L-histidine supplementation).

Based on the above results, one of the most interesting findings is the higher level of taurine in the control group compared to groups fed with a diet containing carnosine synthesis precursor β-alanine. Taurine is a nonproteinogenic amino acid, which is abundant and essential for preserving the integrity and function of the eyes, heart, and skeletal muscles in poultry as well as in the small intestine (He et al., 2021). Given that β-alanine is a taurine transporter inhibitor (Murakami et al., 2010), the small intestine will decrease taurine absorption when the diet contains β-alanine, as shown in our study. Likewise, previous studies have indicated that β-alanine reduces the taurine concentration in the breast muscle and plasma of broilers (Qi et al., 2018). A reduction in intracellular taurine may occur as the elevated β-alanine availability increases the competition of their shared taurine transporter (TauT) (Shaffer and Kocsis, 1981; Kubo et al., 2022). This may cause a carnosine synthesis increase in KR meat that is supplemented with β-alanine in the diet (Suwanvichanee et al., 2022). Similarly, reduced threonine may be involved in physiological and biochemical processes, including growth functions (Ramos-Pinto et al., 2021). Meanwhile, a decrease in betaine level may be involved in energy metabolism and in support cells to maintain their function during stress periods (Awad et al., 2022). As Suwanvichanee et al. (2022) reported that KR can tolerate stress and may not require carnosine as an antioxidant, we suggest that a decrease of both amino acids (threonine and betaine) has no effect on an antioxidant in modulating the growth performance of KR.

Furthermore, when the diet included both carnosine synthesis precursors, the quantities of β-alanine, choline, myo-inositol, and lactate were increased in the jejunum compared with control group (Figure 3.2 B). Choline is important for metabolomic functions and is absorbed in the jejunum and ileum mainly by an energy- and sodium-dependent carrier mechanism (Smolders et al., 2019). In addition, the initial L-histidine uptake rate is reduced by the substitution of Na^+ with various forms of choline in the incubation buffer (Sakurai et al., 2002). Choline deficiency usually decreases feed intake and growth rate in poultry (Navidshad et al., 2018). This may be a reason why, in the previous study by Suwanvichanee et al. (2022), no significant difference in growth performance was found between the group supplemented with the carnosine precursor and the control group.

Lactate is a product of the anaerobic metabolism of glucose (Tanaka et al., 2015). Lactase from ingested lactic acid bacteria may enhance the hydrolysis of lactose to glucose and galactose in the small intestine, which is rapidly absorbed or fermented (Hove et al., 1999). Furthermore, an increased myo-inositol concentration in the small intestine, found in all supplementation groups, is associated with increased myo-inositol concentrations in the plasma of broilers (Sommerfeld et al., 2018). Myo-inositol is endogenously synthesized from D-glucose or generated by the dephosphorylation of intracellular myo-inositol hexakisphosphate (Gonzalez-Uarquin et al., 2020b). It is an important molecule in several chicken metabolic processes (Gonzalez-Uarquin et al., 2020a; Gonzalez-Uarquin et al., 2020b) and improves the performance and health of chickens (Lee and Bedford, 2016). Nevertheless, previous studies found that supplementing the carnosine precursor does not affect production performance in KR (Suwanvichanee et al., 2022). Therefore, these metabolites may play important roles in the overall metabolism involved in carnosine synthesis and may be associated with precursor absorption and transportation in KR jejunum. However, it is important to note that the specific mechanisms of carnosine synthesis, transportation, and utilization in chickens are still an active area of research, and more studies are needed to fully understand the role of carnosine in chickens.

3.4.3 Metabolic Pathway Analysis

Based on the above results, we expected to identify carnosine synthesis-related metabolic pathways, especially β-alanine and L-histidine metabolisms, as the main metabolic pathways affected by β-alanine and L-histidine supplementation.

Thirty-four potential metabolic pathways showed differentiation between the groups (Table 3.3). The results are also shown in the graphical model in Figure 3.3, where the intensity of the red color indicates the significance of the pathway and the size of the circle corresponds to the pathway impact score. Eight pathways had impact values higher than 0.05, $P < 0.05$, and FDR < 0.05 (Table 3.2). These pathways were cysteine and methionine metabolism, glycerophospholipid metabolism, tyrosine metabolism, glycine, serine and threonine metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, phenylalanine metabolism, taurine and hypotaurine metabolism, and pantothenate and CoA biosynthesis. In addition, histidine, β-alanine, and alanine, aspartate, and glutamate metabolisms showed high impact values ($P < 0.05$) with *P*-values of 0.09.

Table 3.3 List of metabolic pathways showing differentiation between the experimental groups based on the enrichment analysis. P-values are adjusted for multiple testing. Pathways with *P*-value < 0.05, FDR < 0.05, and impact > 0.05 are marked in boldface.

No.	Metabolic pathway name	Total[1]	Hits[2]	*P*-Value	FDR[3]	Impact[4]
1	**Cysteine and methionine metabolism**	33	1	0.000099	0.00338	0.10446
2	Valine, leucine, and isoleucine biosynthesis	8	4	0.000263	0.00447	0.00000
3	**Glycerophospholipid metabolism**	35	3	0.000467	0.00489	0.05992
4	Valine, leucine, and isoleucine degradation	40	3	0.000703	0.00489	0.00000
5	Aminoacyl-tRNA biosynthesis	48	13	0.000720	0.00489	0.00000
6	**Tyrosine metabolism**	42	1	0.002907	0.01412	0.13972
7	Ubiquinone and other terpenoid-quinone biosynthesis	9	1	0.002907	0.01412	0.00000
8	**Glycine, serine, and threonine metabolism**	34	4	0.003365	0.01430	0.07063
9	**Phenylalanine, tyrosine, and tryptophan biosynthesis**	4	2	0.006027	0.02049	1.00000
10	**Phenylalanine metabolism**	8	2	0.006027	0.02049	0.35714
11	**Taurine and hypotaurine metabolism**	8	1	0.015473	0.04384	0.42857
12	Primary bile acid biosynthesis	46	1	0.015473	0.04384	0.03799
13	**Pantothenate and CoA biosynthesis**	19	3	0.016845	0.04406	0.07500
14	Butanoate metabolism	15	2	0.042088	0.10221	0.00000
15	Purine metabolism	62	3	0.072628	0.16462	0.02268
16	Histidine metabolism	16	3	0.085022	0.17088	0.22131
17	β-Alanine metabolism	21	3	0.085438	0.17088	0.39925
18	Alanine, aspartate, and glutamate metabolism	28	4	0.092839	0.17536	0.53446
19	Nicotinate and nicotinamide metabolism	15	1	0.122520	0.21925	0.00000
20	Arginine and proline metabolism	38	3	0.186500	0.30901	0.17592
21	Arginine biosynthesis	13	3	0.190860	0.30901	0.07051
22	Glycolysis / Gluconeogenesis	26	1	0.212130	0.31359	0.00000
23	Pyruvate metabolism	22	1	0.212130	0.31359	0.00000
24	D-Glutamine and D-glutamate metabolism	6	2	0.281710	0.36839	0.50000
25	Glyoxylate and dicarboxylate metabolism	32	2	0.281710	0.36839	0.00000
26	Nitrogen metabolism	6	2	0.281710	0.36839	0.00000
27	Pyrimidine metabolism	40	2	0.300360	0.37823	0.00000
28	Inositol phosphate metabolism	30	1	0.504150	0.55293	0.08018
29	Phosphatidylinositol signaling system	28	1	0.504150	0.55293	0.02440
30	Galactose metabolism	27	1	0.504150	0.55293	0.00000
31	Ascorbate and aldarate metabolism	10	1	0.504150	0.55293	0.00000
32	Glutathione metabolism	28	1	0.725930	0.74793	0.01123
33	Porphyrin and chlorophyll metabolism	30	1	0.725930	0.74793	0.00000
34	Propanoate metabolism	23	1	0.797300	0.79730	0.00000

[1] The total number of metabolites in the pathway was obtained from the library.

[2]Number of metabolites actually detected out of 28 metabolites.

[3]False Discovery Rate.

[4]The pathway impact value calculated from pathway topology.

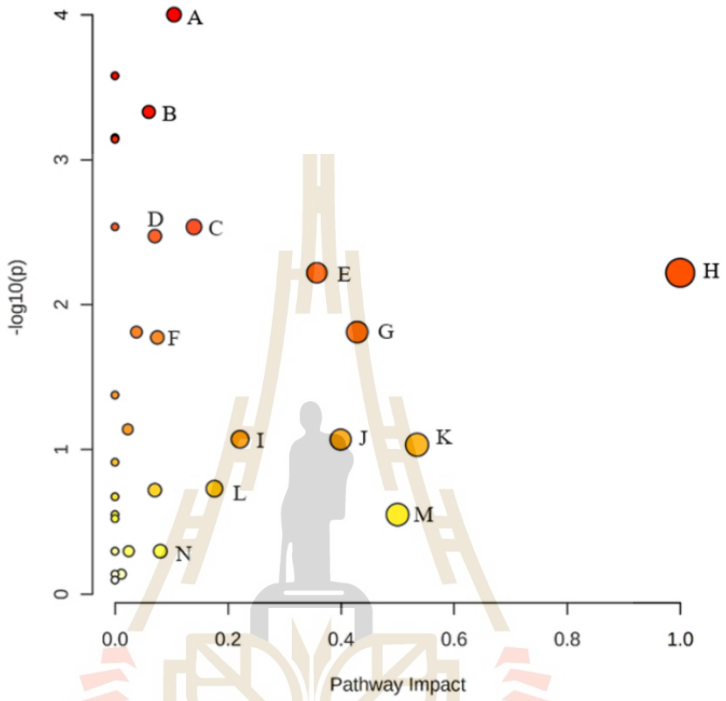

Figure 3.3 Pathway impact values and *P*-values (-$\log_{10}$) from the pathway topology analysis. Color darkness indicates pathway significance (the darker the color the more significant the pathway). (A) Cysteine and methionine metabolism, (B) Glycerophospholipid metabolism, (C) Tyrosine metabolism, (D) Glycine, serine, and threonine metabolism, (E) Phenylalanine metabolism, (F) Pantothenate and CoA biosynthesis, (G) Taurine and hypotaurine metabolism, (H) Phenylalanine, tyrosine, and tryptophan biosynthesis, (I) Histidine metabolism, (J) β-Alanine metabolism, (K) Alanine, aspartate, and glutamate metabolism, (L) Arginine and proline metabolism, (M) D-Glutamine and D-glutamate metabolism, and (N) Inositol phosphate metabolism

Based on previous studies, alanine, aspartate, and glutamate metabolisms are associated with β-alanine and histidine metabolisms and are affected by β-alanine and L-histidine supplementation (Sookoian et al., 2012). In addition, propanoate metabolism, β-alanine metabolism, histidine metabolism, pantothenate and CoA biosynthesis, and alanine metabolism are related to carnosine metabolism (Matthews et al., 2019; Nakata et al., 2020). In chickens, the absorption of carnosine is less efficient than its constituent amino acids L-histidine and β-alanine absorption (Kopec et al., 2020). Previous studies have shown that the breakdown of carnosine into its constituent amino acids occurs rapidly in the small intestine of chickens, with nearly 80% of carnosine being hydrolyzed within 30 minutes of ingestion (Harris et al., 2012). Therefore, the bioavailability of L-histidine and β-alanine may vary depending on the composition of the diet and the digestive physiology of the chicken.

To conclude, even though the differences between the control and the supplementation groups were not statistically significant but had high impact values, carnosine-related pathways, i.e., L-histidine metabolism and β-alanine metabolism, are important in KR jejunum. In addition, we found other pathways involved in β-alanine, L-histidine, and carnosine metabolism, e.g., alanine metabolism. The impact of these pathways on metabolic pathways depends on the role of the pathway in the overall metabolism and its function in the organism (Matthews et al., 2019; Nakata et al., 2020).

3.4.4 The Intensity Ratios of Biomolecules and the Secondary Protein Structure using SR-FTIR

Synchrotron radiation-based Fourier transform infrared microspectroscopy techniques were used to confirm the molecular structural and biochemical changes in the jejunum.

Significant differences in SR-FTIR spectra were obtained in the regions of amide I, II, III, and CH-bending ($P < 0.05$) (Table 3.4, Figures 3.4A and B). The integral area of amide I in group B was higher than in the other groups ($P < 0.05$), whereas the control group and L-histidine precursor group did not show significant differences. Related to amide II, the integral areas in group B were higher than in the control group and the β-alanine and L-histidine precursor group ($P < 0.05$) but were not significantly different from the L-histidine precursor group. The β-alanine and L-histidine precursor

group showed higher integral areas of CH-bending and amide III than the control, β-alanine, and L-histidine precursor groups ($P < 0.05$). Regarding CH-bending, the integral areas of the L-histidine precursor group were lower than in the control group, but the β-alanine precursor group was not significantly different from the control group. In addition, glycogen/carbohydrate showed non-significant differences among four groups ($P > 0.05$).

Table 3.4 Integral areas of the SR-FTIR spectra averaged over 200 spectra per experimental group.

Biomolecule (Wavenumber)	Experimental groups[1]				SEM[2]	*P*-value
	A	B	C	D		
Amide I (1700–1600 cm^{-1})	0.0070^{b}	0.0080^{a}	0.0070^{b}	0.0064^{c}	0.0001	< 0.001
Amide II (1600–1500 cm^{-1})	0.0020^{b}	0.0034^{a}	0.0026^{ab}	0.0022^{b}	0.0003	0.007
CH-bending (1450–1390 cm^{-1})	0.0008^{b}	0.00001^{c}	0.0010^{b}	0.0038^{a}	0.0002	< 0.001
Amide III (1320–1220 cm^{-1})	0.0010^{b}	0.0010^{b}	0.0006^{b}	0.0032^{a}	0.0002	< 0.001
Glycogen/Carbohydrate (1200–900 cm^{-1})	0.0012	0.0020	0.0016	0.0016	0.0005	0.450

[1]Diet groups. A: control, B: supplementation with 1.0% β-alanine, C: supplementation with 0.5% L-histidine, D: supplementation with 1.0% β-alanine and 0.5% L-histidine

[2]Standard error of mean

a–c Significantly different treatment groups with *P*-value < 0.05.

Our results suggest that carnosine synthesis precursors strongly affect amide I, II, and III and CH-bending in the chicken jejunum. Previous studies (Yu and Nuez-Ortín, 2010) indicate that the amide I to amide II ratio has a positive correlation with the metabolizable protein, while the slowly degradable fraction of protein is positively correlated with the amide I level. The amide I band, which is sensitive to small differentiation in molecular structure and hydrogen bonding motifs, is important for determining protein structural and conformational changes (Ismael et al., 2019). Therefore, it is possible that the group of β-alanine precursor may affect amide I protein transformation because amide I can provide information on the vibrational bands of the protein backbone (Sadat and Joye, 2020). Moreover, amide III levels were high

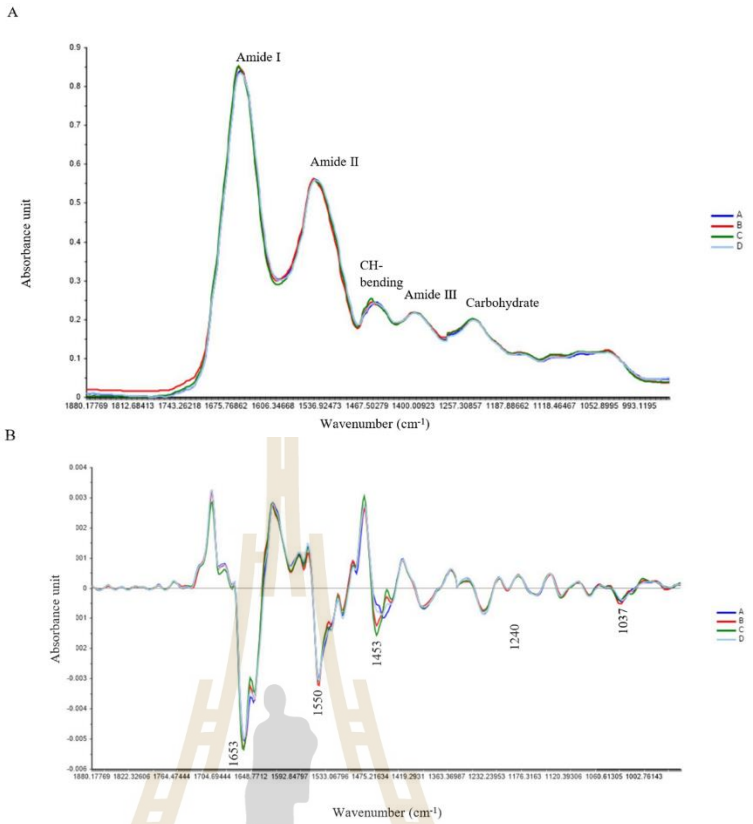

Figure 3.4 Averages of the original (A) and second-derivative (B) spectra values in the 1700 –800 cm^{-1} regions of the experimental groups; A (control diet), B (diet with 1.0% β-alanine supplementation), C (diet with 0.5% L-histidine supplementation), and D (diet with 1.0% β-alanine and 0.5% L-histidine supplementation).

in the group of β-alanine and L-histidine precursors, characterized by changes in collagen secondary structures and CH-bending (Dehring et al., 2006). The CH-bending in β-alanine and L-histidine refers to the movement of the hydrogen atoms attached to the carbon atoms in these amino acids and contains CH bonds that can undergo bending motions (Freire et al., 2017). Likewise, CH-bending in β-alanine and in carnosine can have important implications to the biological activity of these molecules and to their interactions with other molecules in the body (Matthews et al., 2019). Accordingly, CH-bending was found to be high in the β-alanine precursor group.

Moreover, to determine the secondary structure of proteins, we calculated second derivatives of the obtained FTIR spectra and made a deconvolution of amide I at the IR region. The proportion of different protein secondary structures in the amide I region is presented in Table 3.5. Significant differences between the experimental groups were obtained for the β-sheet and α-helix ($P < 0.05$). The content of β-sheet in the β-alanine precursor group and in the L-histidine precursor group was higher than in the control group or in the β-alanine and L-histidine precursor group. In addition, the α-helix content in the β-alanine precursor group was lower than that in the control group.

Table 3.5 Relative proportion (%) of secondary protein structures in the amide I region averaged over 200 spectra per experimental group.

Secondary protein structure (Wavenumber)	Experimental group[1]				SEM[2]	*P*-value
	A	B	C	D		
β-sheet (1630 cm^{-1})	25.12[b]	27.06[a]	27.21[a]	25.51[b]	0.421	< 0.001
α-helix (1644 cm^{-1}, 1655 cm^{-1})	34.75[a]	32.19[b]	33.27[ab]	33.67[ab]	0.621	0.007
β-turn (1670 cm^{-1})	18.95	18.15	18.38	18.95	0.497	0.296
Antiparallel (1689 cm^{-1})	21.18	22.60	21.14	21.86	0.661	0.132

[1]Diet groups. A: control, B: supplementation with 1.0% β-alanine, C: supplementation with 0.5% L-histidine, D: supplementation with 1.0% β-alanine and 0.5% L-histidine.
[2]Standard error of mean.
a–c Significantly different treatment groups with *P*-value < 0.05.

Our results confirmed that carnosine synthesis precursor supplements in the diet influence amide I and protein secondary structures in the jejunum; namely the β-alanine precursor group increases the β-sheet and decreases the α-helix content in chicken jejunum. β-alanine precursor supplementation in the diet has also been shown to have a beneficial effect and to diminish tissue damage in the small intestine in rats (Brencher et al., 2017). In addition, elevating the β-sheet to an α-helix ratio can increase the digestible and indigestible protein fraction in animals, and a high

percentage of the β-sheet in amide I may cause low access to digestive enzymes in the gastrointestinal tract (Yu, 2004).

Consistent with previous studies by Abbasi et al. (2014), Cui et al. (2020), and Qiu et al. (2022), we have shown that carnosine precursor supplementation in the diet may affect the biochemical compounds in the jejunum.

3.4.5 PCA Analysis of Biochemical Compounds, Protein Secondary Structures and Metabolite Content

Principal component analysis was used to confirm the dependence between the metabolites that had a VIP value > 1 and both the biochemical compounds and protein secondary structures. The score plot and correlation loadings related to SR-FTIR spectra, secondary structure, and metabolite content are shown in Figures 3.5A and B. These plots show that the first two principal components (**PCs**) carry a large amount of information, explaining 66% of the data variance, with PC1 explaining the most (45%) and PC2 explaining much less variance (21%). The plot contains two ellipses that indicate how much variance is considered; the outer ellipse indicates 100% of the explained variance and the inner ellipse indicates 50% of the explained variance. Variables that are found between the two ellipses, and particularly those positioned near the edge of the outer ellipse, are those that are most important in the differentiation. All variables located in the outer circle region (α-helix, amide I, II, III, CH-bending, creatine, tyrosine, valine, isoleucine, aspartate, β-alanine, choline, myo-inositol, and lactate) were significantly correlated with different experimental groups; the control group was positively correlated with α-helix, while the β-alanine precursor group, and the L-histidine precursor group were highly positively correlated with amide I, II, creatine, tyrosine, valine, isoleucine, and aspartate, and the β-alanine and L-histidine precursor group was positively correlated with amide III and CH-bending.

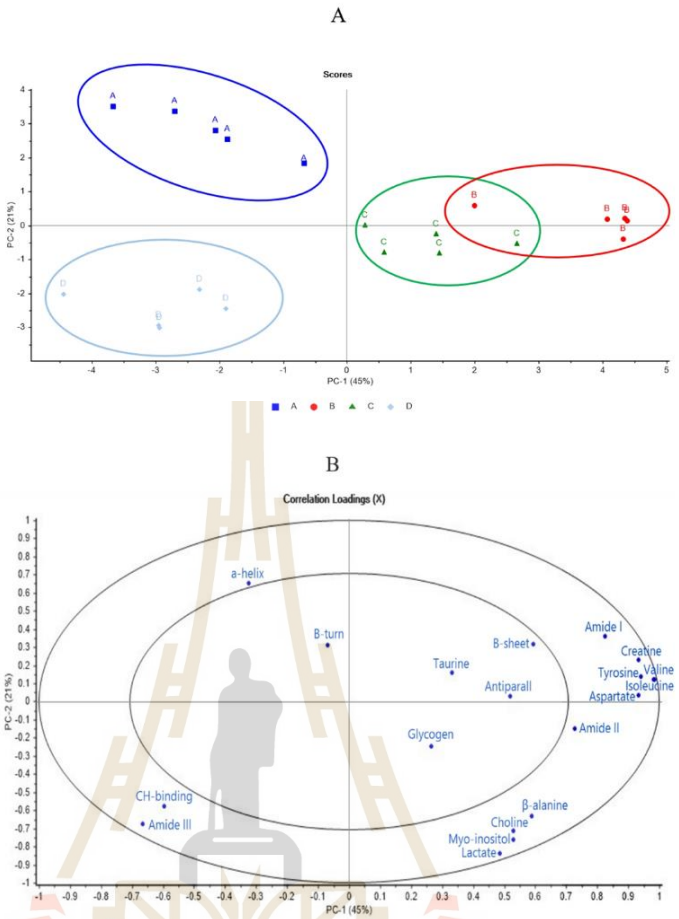

Figure 3.5 Principal component (PC) analysis score plot (A) for PC1 versus PC2 of the different experimental groups and correlation loading plot (B) for PC1 versus PC2. The PCs explain 66% of the total variance. Experimental groups; A (control diet), B (diet with 1.0% β-alanine supplementation), C (diet with 0.5% L-histidine supplementation), and D (diet with 1.0% β-alanine and 0.5% L-histidine supplementation).

The results indicate signaling or metabolic pathways that are involved in the absorption and transportation of carnosine synthesis precursors. Overall, these metabolites can be used for determining the biochemical compound and protein structure changes and for monitoring precursor responses in the jejunum.

3.5 Conclusions

In this study, we investigated the metabolic and biochemical compound changes in KR jejunum caused by β-alanine and L-histidine supplementation in the diet. We identified metabolite contents and metabolic pathways that could be linked to these amino acid precursors. The 10 key metabolites that differentiated the diet groups were β-alanine, choline, myo-inositol, creatine, lactate, aspartate, tyrosine, isoleucine, valine, and taurine. The relative proportion of different secondary protein structures indicated that the β-alanine precursor in the diet increases β-sheets and decreases α-helix in the amide I region in jejunal tissue. In addition, an L-histidine precursor in the diet increases β-sheets in the amide I region. Principal component analysis revealed that a diet with both precursors of the carnosine synthesis is strongly and positively associated with amide I, amide II, creatine, tyrosine, valine, isoleucine, and aspartate levels in the jejunum. Therefore, our study confirmed that precursor supplements in the diet influence biochemical changes in jejunal tissue and can be used for monitoring the precursor response. However, a deeper understanding of the interplay between metabolites and transcriptome may provide insights into the mechanisms underlying cellular functions and precursor responses in the jejunum. Thus, the natural next step is to study gene expression in jejunal tissue under a diet with and without carnosine synthesis precursors.

3.6 References

Abbasi, M. A., Mahdavi, A. H., Samie, A. H., and Jahanian, R. (2014). Effects of different levels of dietary crude protein and threonine on performance, humoral immune responses and intestinal morphology of broiler chicks. **Brazilian Journal of Poultry Science**. 16(1). 35- 44.

Abreu, A. C., and Fernández, I. (2020). NMR metabolomics applied on the discrimination of variables influencing tomato (Solanum lycopersicum). **Molecules**. 25(16). 3738.

Awad, W. A., Ruhnau, D., Gavrău, A., Dublecz, K., and Hess, M. (2022). Comparing effects of natural betaine and betaine hydrochloride on gut physiology in broiler chickens. **Poultry Science**. 101(12). 102173.

Blancquaert, L., Everaert, I., Missinne, M., Baguet, A., Stegen, S., Volkaert, A., and Derave, W. (2017). Effects of histidine and β-alanine supplementation on human muscle carnosine storage. **Med Sci Sports Exerc**. 49(3). 602-609.

Brencher, L., Verhaegh, R., and Kirsch, M. (2017). Attenuation of intestinal ischemia-reperfusion-injury by β-alanine: a potentially glycine-receptor mediated effect. **Journal of Surgical Research**. 211. 233-241.

Cui, Z., Tan, B., Liao, S., Qi, M., Wang, X., Zha, A., and Liao, P. (2020). Dietary addition of antioxidant complex packs and functional amino acids can improve the digestion, absorption, and immunity of huanjiang minipigs. **BioMed Research International**. 2020.

Dehring, K. A., Smukler, A. R., Roessler, B. J., and Morris, M. D. (2006). Correlating changes in collagen secondary structure with aging and defective type II collagen by Raman spectroscopy. **Applied spectroscopy**. 60(4). 366-372.

Derave, W., Everaert, I., Beeckman, S., and Baguet, A. (2010). Muscle carnosine metabolism and β-alanine supplementation in relation to exercise and training. **Sports medicine**. 40(3). 247-263.

Ferraris, R. P., Diamond, J. A. R. E. D., and Kwan, W. W. (1988). Dietary regulation of intestinal transport of the dipeptide carnosine. **American Journal of Physiology-Gastrointestinal and Liver Physiology**. 255(2). G143-G150.

Freire, P. T., Barboza, F. M., Lima, J. A., Melo, F. E., and Mendes Filho, J. (2017). Raman spectroscopy of amino acid crystals. **Raman Spectroscopy and Applications**. 201. 171.

Gonzalez-Uarquin, F., Rodehutscord, M., and Huber, K. (2020a). Myo-inositol: Its metabolism and potential implications for poultry nutrition—A review. **Poultry Science**. 99(2). 893-905.

Gonzalez-Uarquin, F., Molano, E., Heinrich, F., Sommerfeld, V., Rodehutscord, M., and Huber, K. (2020b). Research Note: Jejunum phosphatases and systemic myo-inositol in broiler chickens fed without or with supplemented phytase. **Poultry Science**. 99(11). 5972-5976.

Harris, R. C., Wise, J. A., Price, K. A., Kim, H. J., Kim, C. K., and Sale, C. (2012). Determinants of muscle carnosine content. **Amino acids**. 43. 5-12.

He, W., Li, P., and Wu, G. (2021). Amino acid nutrition and metabolism in chickens. **In Amino Acids in Nutrition and Health** (pp. 109-131). Springer, Cham.

Hipkiss, A. R., Cartwright, S. P., Bromley, C., Gross, S. R., and Bill, R. M. (2013). Carnosine can understanding its actions on energy metabolism and protein homeostasis inform its therapeutic potential?. **Chemistry Central Journal**. 7(1): 38.

Hove, H., Nørgaard, H., and Brøbech Mortensen, P. (1999). Lactic acid bacteria and the human gastrointestinal tract. **European Journal of Clinical Nutrition**. 53(5). 339-350.

Ismael, A., Guevara-Oquendo, V. H., Refat, B., and Yu, P. (2019). Interactive association between processing induced molecular structure changes and nutrient delivery on a molecular basis, revealed by cutting-edge vibrational biomolecular spectroscopy. **Journal of animal science and biotechnology**. 10(1). 1-16.

Jukić, I., Kolobarić, N., Stupin, A., Matić, A., Kozina, N., Mihaljević, Z., and Drenjančević, I. (2021). Carnosine, small but mighty—Prospect of use as functional ingredient for functional food formulation. **Antioxidants**. 10(7). 1037.

Kopec, W., Jamroz, D., Wiliczkiewicz, A., Biazik, E., Pudlo, A., Korzeniowska, M., and Skiba, T. (2020). Antioxidative characteristics of chicken breast meat and blood after diet supplementation with carnosine, L-histidine, and β-alanine. **Antioxidants**. 9(11). 1093.

Kubo, Y., Ishizuka, S., Ito, T., Yoneyama, D., Akanuma, S. I., and Hosoya, K. I. (2022). Involvement of TauT/SLC6A6 in taurine transport at the blood–testis barrier. **Metabolites**. 12(1). 66.

Kubota, S., Promkhun, K., Sinpru, P., Suwanvichanee, C., Molee, W., and Molee, A. (2021). RNA profiles of the Korat chicken breast muscle with increased carnosine content produced through dietary supplementation with β-alanine or l-histidine. **Animals**. 11(9). 2596.

Lackner, J., Hess, V., Marx, A., Hosseini-Ghaffari, M., and Sauerwein, H. (2022). Effects of dietary supplementation with histidine and β-alanine on blood

plasma metabolome of broiler chickens at different ages. **Plos one**. 17(11). e0277476.

Le Roy, C. I., Mappley, L. J., La Ragione, R. M., Woodward, M. J., and Claus, S. P. (2016). NMR-based metabolic characterization of chicken tissues and biofluids: a model for avian research. **Metabolomics**. 12(10). 1-14.

Lee, S. A., and Bedford, M. R. (2016). Inositol-An effective growth promotor?. **World's Poultry Science Journal**. 72(4). 743-760.

Matthews, J. J., Artioli, G. G., Turner, M. D., and Sale, C. (2019). The physiological roles of carnosine and β-alanine in exercising human skeletal muscle. **Medicine & Science in Sports & Exercise**. 51(10). 2098-2108.

Murakami, T., and Furuse, M. (2010). The impact of taurine-and beta-alanine-supplemented diets on behavioral and neurochemical parameters in mice: antidepressant versus anxiolytic-like effects. **Amino acids**. 39(2). 427-434.

Nakata, R., Sato, M., and Tomonaga, S. (2020). Effects of dietary defatted meat species on metabolomic profiles of murine liver, gastrocnemius muscle, and cecal content. **Metabolites**. 10(12). 503.

Navidshad, B., Maghsoodi, Z., Nikbin, S., Vahedi, V., Adibmoradi, M., Mirzaei Aghjehgheshlagh, F., and Moradi, H. (2018). The compensation effect of dietary garlic on chicken consuming a minimal level of choline. **Italian Journal of Animal Science**. 17(1). 175-179.

Perim, P., Marticorena, F. M., Ribeiro, F., Barreto, G., Gobbi, N., Kerksick, C., and Saunders, B. (2019). Can the skeletal muscle carnosine response to beta-alanine supplementation be optimized?. **Frontiers in nutrition**. 135.

Poompramun, C., Molee, W., Thumanu, K., and Molee, A. (2021). The significant influence of residual feed intake on flavor precursors and biomolecules in slow-growing Korat chicken meat. **Animal Bioscience**. 34(10). 1684.

Qi, B., Wang, J., Ma, Y. B., Wu, S. G., Qi, G. H., and Zhang, H. J. (2018). Effect of dietary β-alanine supplementation on growth performance, meat quality, carnosine content, and gene expression of carnosine-related enzymes in broilers. **Poultry science**. 97(4). 1220-1228.

Qiu, Y., Gao, X., Chen, R., Lu, S., Wan, X., Farag, M. A., and Zhao, C. (2022). Metabolomics and biochemical insights on the regulation of aging-related

diabetes by a low-molecular-weight polysaccharide from green microalga Chlorella pyrenoidosa. **Food Chemistry: X**. 14. 100316.

Ramos-Pinto, L., Machado, M., Calduch-Giner, J., Pérez-Sánchez, J., Dias, J., Conceição, L. E., and Costas, B. (2021). Dietary histidine, threonine, or taurine supplementation affects gilthead seabream (Sparus aurata) immune status. **Animals**. 11(5). 1193.

Sadat, A., and Joye, I. J. (2020). Peak fitting applied to fourier transform infrared and raman spectroscopic analysis of proteins. **Applied Sciences**. 10(17). 5918.

Sadikali, F., Darwish, R., and Watson, W. C. (1975). Carnosinase activity of human gastrointestinal mucosa. **Gut**. 16(8). 585-589.

Sakurai, E., Sakurada, T., Ochiai, Y., Yamakami, J., and Tanaka, Y. (2002). Stereoselective transport of histidine in rat lung microvascular endothelial cells. **American Journal of Physiology-Lung Cellular and Molecular Physiology**. 282(6). L1192-L1197.

Sale, C., Artioli, G. G., Gualano, B., Saunders, B., Hobson, R. M., and Harris, R. C. (2013). Carnosine: from exercise performance to health. **Amino acids**. 44(6). 1477-1491.

Shaffer, J. E., and Kocsis, J. J. (1981). Taurine mobilizing effects of beta-alanine and other inhibitors of taurine transport. **Life Sciences**. 28(24). 2727-2736.

Smolders, L., de Wit, N. J., Balvers, M. G., Obeid, R., Vissers, M. M., and Esser, D. (2019). Natural choline from egg yolk phospholipids is more efficiently absorbed compared with choline bitartrate; outcomes of a randomized trial in healthy adults. **Nutrients**. 11(11). 2758.

Sommerfeld, V., Schollenberger, M., Kühn, I., and Rodehutscord, M. (2018). Interactive effects of phosphorus, calcium, and phytase supplements on products of phytate degradation in the digestive tract of broiler chickens. **Poultry Science**. 97(4). 1177-1188.

Sookoian, S., and Pirola, C. J. (2012). Alanine and aspartate aminotransferase and glutamine-cycling pathway: their roles in pathogenesis of metabolic syndrome. **World journal of gastroenterology: WJG**. 18(29). 3775.

Suwanvichanee, C., Sinpru, P., Promkhun, K., Kubota, S., Riou, C., Molee, W., and Molee, A. (2022). Effects of β-alanine and L-histidine supplementation on

carnosine contents in and quality and secondary structure of proteins in slow-growing Korat chicken meat. **Poultry Science**. 101(5). 101776.

Tanaka, K., Hashimoto, H., and Ohki, T. (2015). Lactate levels in bowel strangulation with experimental animal model. **International surgery**. 100(2). 240-243.

Xiao, Z., Ge, C., Zhou, G., Zhang, W., and Liao, G. (2019). 1H NMR-based metabolic characterization of Chinese Wuding chicken meat. **Food chemistry**. 274. 574-582.

Xing, L., Chee, M. E., Zhang, H., Zhang, W., and Mine, Y. (2019). Carnosine—A natural bioactive dipeptide: Bioaccessibility, bioavailability and health benefits. **Journal of Food Bioactives**. 5. 8-17.

Yu, P. (2004). Application of advanced synchrotron radiation-based Fourier transform infrared (SR-FTIR) microspectroscopy to animal nutrition and feed science: a novel approach. **British Journal of nutrition**. 92(6). 869-885.

Yu, P., and Nuez-Ortín, W. G. (2010). Relationship of protein molecular structure to metabolisable proteins in different types of dried distillers grains with solubles: a novel approach. **British Journal of Nutrition**. 104(10). 1429-1437.

CHAPTER IV

JEJUNAL TRANSCRIPTOMIC PROFILING OF CARNOSINE SYNTHESIS PRECURSORS RELATED GENES AND PATHWAYS IN SLOW-GROWING KORAT CHICKEN

4.1 Abstract

The slow-growing Korat chicken (**KR**) is known for its high carnosine content in breast meat that can be further increased by dietary supplementation of carnosine synthesis precursors β-alanine and L-histidine. However, the genes and biological pathways of β-alanine and L-histidine absorption and transportation in slow-growing chickens are not yet studied. Our objective was to advance understanding of the β-alanine and L-histidine absorption and transportation caused by supplementation of β-alanine and L-histidine in diet and revealed the pathways and genes through jejunal transcriptomic profiling. Whole jejunum samples from 5 control and 5 experimental KR chicken, fed with 1% β-alanine and 0.5% L-histidine supplementation, were collected. A total of 407 differentially expressed genes ($P < 0.05$, $\log_2$ fold change ≥ 2) were identified including *KCND3, OPRM1, CCK, GCG, TRH,* and *GABBR2* as the key genes by pathway enrichment analysis and protein-protein interaction (PPI) network. A total of 272 genes were down-regulated and 135 up-regulated in a group with diet supplementation compared to a control group. Eighty-seven gene ontology terms were identified and six KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways were significantly (*P*- adjust < 0.05) enriched in the jejunum through integrated analysis of the protein-protein interaction network and KEGG pathway maps. These findings give insight of the molecular mechanism related to carnosine precursor absorption and transportation in the jejunum and help to identify useful molecular markers for improvement of the carnosine content in slow-growing KR chicken meat.

Keywords: carnosine precursor, β-alanine, L-histidine, jejunal tissue, transcriptomic

4.2 Introduction

Different breed lines between a male line, Leung Hang Khao Thai indigenous chicken, and a female line, SUT (Suranaree University of Technology) were selected for more than 10 generations and mated to produce crossbred, the slow-growing Korat chicken (KR). KR is a slow-growing chicken (average daily gain is 19.8–21.0 g/day) (Sinpru et al., 2021) and has been developed for smallholder farmers in Thailand. KR is known for its tasty meat with rich flavor and unique texture (Katemala et al., 2021). In addition, KR meat has a high carnosine content (Suwanvichanee et al., 2022). However, female KR is not required by farmers because they grow slower rate than male KR, which takes a longer time to raise than male KR but can sell at the same price. Thus, it would be ideal to increase the value of female KR.

A previous study reported that female Thai indigenous chicken meat contains higher levels of carnosine than males (Intarapichet and Maikhunthod, 2005). Likewise, our previous study found that female KR breast meat had a high carnosine content when fed with dietary supplementation with β-alanine and L-histidine (Suwanvichanee et al., 2022). These findings imply that female KR with increased carnosine content produced through supplemented diets can be made more attractive to the consumers demand as a functional food, which inspires farmers' readiness to raise female KR.

Carnosine content in meat can be increased by absorption of carnosine synthesis precursors β-alanine and L-histidine through diet supplementation (Kopec et al., 2020; Lackner et al., 2021; Suwanvichanee et al., 2022). The increase of carnosine in muscle is predominantly due to the ingestion of histidine-containing dipeptides such as carnosine, anserine, and balenine or β-alanine (Bonfanti et al., 1999; Miyaji et al., 2012). Ingestion of β-alanine or carnosine in muscle is degraded by carnosinase, and synthesized by carnosine synthase (Miyaji et al., 2012). Previous studies reported that carnosine is easily absorbed in the gastrointestinal tract and enters portal circulation (Xing et al., 2019) and jejunum has the highest ability to absorb carnosine compared with duodenum and ileum because of high activity of carnosinase enzyme that acts against the carnosine substrate the jejunal mucosa (Sadikali et al., 1975; Ferraris et al., 1988; Sale et al., 2013). As an example, carnosine content in female KR breast meat was 52.8% higher in a group that was supplemented with β-alanine and L-histidine

compared to the control group without supplementation without any adverse effect on growth performance, meat quality, and texture (Suwanvichanee et al., 2022). Furthermore, our previous studies have performed transcriptomic analysis to reveal the molecular mechanisms related to carnosine synthesis in KR breast meat, they found the potential genes (i.e., *MYO7B, MYBPH, LOC107051274, SERPINH1, ACSBG1, CAPNS2,* and *PGAM1*) related to meat tenderness but could not identify the carnosine synthase gene. Moreover, the functional enrichment pathway affected by carnosine precursor supplementation was identified, including insulin signaling pathway, the insulin resistance and adipocytokine signaling pathways and FoxO signaling pathway (Kubota et al., 2021). Recently, based on the jejunum metabolite profile analysis, chicken fed with β-alanine and L-histidine supplementation had higher concentrations of β-alanine, butyrate, choline, creatine, glutamate, glutamine, lactate, methionine, and myo-inositol and lower concentrations of betaine, threonine, and taurine compared to chicken without β-alanine and L-histidine diet supplementation and we also identified the metabolic pathways that could be linked to carnosine precursor (Promkhun et al., 2023). However, carnosine synthesis can be influenced by many factors such as sex (Everaert et al., 2013), age (Kralik et al.,2015; Everaert et al., 2013), breed (Maikhunthod and Intarapichet, 2005; Jung et al., 2013), diet (Tomonaga et al., 2012; Kai et al., 2015; Kralik et al., 2015) and also the ability to absorb and transport amino acids precursor (Xing et al., 2019) which were considered limiting factors for carnosine synthesis in chickens (Brosnan and Brosnan, 2020). Unfortunately, no studies have been conducted on gene expression and the molecular mechanisms related to carnosine precursors β-alanine and L-histidine absorption and transportation in chicken jejunum. Therefore, our research question would be elucidated by gene differentiation and biological mechanisms behind carnosine synthesis related to β-alanine and L-histidine absorption and transportation in jejunum chicken through dietary supplementation with β-alanine and L-histidine.

Our main interest in this study is genes and pathways related to absorption and transportation of carnosine synthesis precursors, its constituent amino acids, and the activities of peptide transporters, β-alanine transporters, and transaminases in chicken jejunum. The mechanism of carnosine synthesis in chicken can be improved by

supplementation of the diet with the carnosine synthesis precursor (Cararo et al., 2015; Jukić et al., 2021). Previous studies have reported that the metabolic pathway of carnosine is regulated by carnosine synthase gene (*CARNS*) (Stenesh and Winnick, 1960) and carnosinase activity genes *CN1* and *CN2* (Creighton et al., 2022). The *CN2* gene is less expressed in kidney and liver, its presence has been evidenced in small intestine, and part of the absorbed carnosine can be hydrolyzed within the enterocyte (Bauchart et al., 2007; Blancquaert et al., 2016) and intact carnosine is absorbed in the jejunum by a specific active transport mechanism (Maikhunthod and Intarapichet, 2005). Additionally, β-alanine and L-histidine are transported with high affinity by the sodium- and chloride-dependent β-alanine carrier (Munck and Munck, 1994) into jejunal brush-border membrane vesicles, particularly in the jejunal mucosa (Sadikali et al., 1975). Moreover, the proton-coupled amino acid transporter (*PAT1*) and sodium and chloride-dependent taurine transporter (*TauT*) are likely to facilitate β-alanine transportation, and the proton-coupled oligopeptide transporter family (peptide transporter 1 and 2; *PEPT1* and *PEPT2*), peptide-histidine transporter 1 and 2 (*PHT1* and *PHT2*), collectively known as the solute carrier family 15 (*SLC15*), are responsible for cellular transportation of peptides (Creighton et al., 2022). Currently, the mechanisms underlying the intact absorption and transportation of βA and L-His across the jejunum in slow-growing chickens remain unknown. Thus, further research is required to unravel the intricate details of these processes and to enhance our understanding of amino acid absorption and transportation in slow-growing chicken.

Therefore, the objective of our study is to use transcriptome analysis to advance the current understanding of biological mechanisms associated with carnosine precursors β-alanine and L-histidine absorption and transportation in KR chicken jejunum achieved through carnosine precursors supplementation in the diet. The genes involved in the carnosine process can be used in the KR breeding program to improve competitiveness in the Thai chicken industry.

4.3 Materials and methods

4.3.1 Animals, Sample Collection and Experimental

All animal protocols used in this research were approved by the Ethics Committee on Animal Use of the Suranaree University of Technology, Nakhon Ratchasima, Thailand (permit number SUT-IACUC-018/2017).

We used 10 whole jejunum samples (five replications per group) of female KR collected in a previous study by Suwanvichanee et al. (2022). In this study, the samples were separated into 2 groups: the control group (basal diet without supplements) and the carnosine synthesis precursors group (a diet supplemented with 1% (1 g/100 g diet) β-alanine (Sigma-Aldrich, St. Louis, MO, USA, 146064) and 0.5% (0.5 g/100 g diet) L-histidine (AppliChem GmbH, Darmstadt, Germany, A3738). The experimental setting was based on findings by Suwanvichanee et al. (2022) where significantly higher carnosine content and better meat characteristics were found in a group fed with the same experimental diet as in this study compared to a control group. In addition, Promkhun et al. (2023) found that a group fed with 1.0% β-alanine and 0.5% L-histidine supplement in diet had significant differences in metabolite concentration and biochemical composition in jejunum tissue compared to control group (both groups same as in this study). At 4 to 10 weeks of age, the chickens in the experimental group were fed with a diet containing 1% β-alanine and 0.5% L-histidine following the detailed feeding management design, raising conditions, and chickens sampling (for more information see Suwanvichanee et al. (2022)).

For transcriptome analysis, chickens at 10 weeks of age were slaughtered, and 10 whole jejunal tissue was extracted. The tissues were collected in tubes, frozen in liquid nitrogen immediately, and kept at −80°C until analysis.

4.3.2 RNA Extraction

The whole jejunal tissue from each chicken was lysed and homogenized in TRIzol reagent (Thermo Fisher Scientific, Waltham, MA). After adding 200 µl of Chloroform-isoamyl alcohol, 24:1 (v/v) the samples were incubated for 5 min at 25°C. The samples were then centrifuged at 12,000 g for 10 min at 4°C (Thermo Fisher Scientific, Langenselbold, Germany), and the supernatants were transferred to new tubes, incubated with chloroform for 5 min at 4°C and centrifuged. After that, the

supernatants were removed and the pellets were precipitated using 50 µl of the 3M sodium acetate and 500 µl of isopropanol, washed with 75% ethanol, and dried for 10 min at 25°C. RNA pellets were resuspended by nuclease-free water. The quantity and quality of the total RNA extraction were checked on 1% agarose gel electrophoresis and measured by NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). All samples with RNA integrity number (RIN) values above 7 were used for the library preparation.

4.3.3 RNA Sequencing Library Construction

Library construction and sequencing were performed by Vishuo Biomedical Ltd. (Bangkok, Thailand) according to the manufacturer's protocol (NEBNext®Ultra™ RNA Library Prep Kit for Illumina®; Illumina, New England Biolabs Inc., Ipswich, MA). For RNA-seq preparation, isolate poly(A) was performed using the NEBNext® Poly(A) mRNA Magnetic Isolation Module (New England BioLabs, Ipswich, MA). Fragmentation and priming the mRNA were performed using NEBNext® First Strand Synthesis Reaction Buffer and Random Primers (New England BioLabs, Ipswich, MA). First-strand and second-strand cDNA were synthesized using ProtoScript® II Reverse Transcriptase (New England BioLabs, MA) and the Second Strand Synthesis Enzyme Mix (New England BioLabs, MA), respectively. Purified double-stranded cDNA was produced using NEBNext End Prep Enzyme Mix (New England BioLabs, MA) to repair both ends and add a dA-tailing in one reaction, followed by a T-A ligation to add adaptors to both ends. Adaptor-ligated DNA was then selected using beads, and fragments of ~400 bp with an insert size of approximately 300 bp were recovered. Each sample was then amplified by polymerase chain reaction (PCR) for 13 cycles. The PCR products were purified using beads, validated, and quantified using a Qsep100 system (BiOptic, New Taipei City, Taiwan) or Qubit 3.0 fluorometer (Invitrogen, Carlsbad, CA), respectively. Libraries were analyzed using an Illumina Novaseq 6000 instrument (Illumina, San Diego, CA) according to the manufacturer's instructions. The libraries were sequenced using paired-end configurations with a read length of 2 × 150 bp on an Illumina HiSeq X instrument (Illumina, San Diego, CA). Base calling was performed using the NovaSeq software (Kubota et al., 2021).

4.3.4 Processing and Analysis of Gene Expression Analysis

RNA sequence reads were obtained from the sequencing and filtered to remove the adapter and low-quality reads (Martin, 2011). High-quality data (clean reads) were generated for further analysis. Hisat2 version 2.0.5 software (Kim et al., 2013) was used to align the clean reads against the chicken reference genome GRCg6a (GenBank Assembly ID: GCA_000002315.5).

The expression levels were normalized using Fragment Per Kilo bases per Million reads method (Trapnell et al., 2010) to find differentially expressed genes (**DEGs**) between the control group and the carnosine synthesis precursors group. The differences between the expression levels were calculated by using the DESeq2 R version 1.20.0 package in R (version 3.22.5) software. Gene expression differences with the *P*-value < 0.05 and $\log_2$ fold change (**FC**) ≥ 2 between the two groups were considered as significant DEGs.

4.3.5 Gene Ontology and Pathway Enrichment Analysis

Functions of the DEGs were predicted using the Ensembl and Entrez Gene (NCBI) databases for *Gallus gallus* and ViSEAGO software (Brionne et al., 2019) in R. Functional enrichment analysis was performed for DEGs with $P < 0.05$ using Gene Ontology (**GO**) and Kyoto Encyclopedia of Genes and Genomes (**KEGG**) database. Three terms in the GO database were mapped: biological process, cellular component, and molecular function. In addition, the Pathway database within KEGG database was used to identify the pathways involved in the DEGs to understand biological functions of these genes. Finally, STRING database (http://string-db.org/ (Franceschini et al., 2013)) was used to explore the interaction between the DEGs.

4.3.6 Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR) Analysis

The RNA-seq results were validated by RT-qPCR. The total RNA was reverse-transcribed into first-stand cDNA using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Carlsbad, CA) according to the manufacturer's guidelines. Six transcripts were randomly selected from the DEGs (potassium voltage-gated channel subfamily D member 3 (*KCND3*), opioid receptor mu 1 (*OPRM1*), cholecystokinin (*CCK*), glucagon (*GCG*), thyrotropin-releasing hormone (*TRH*), and

gamma-aminobutyric acid type B receptor subunit 2 (*GABBR2*)) and primers were designed using Primer3 primer-design software (https://primer3.ut.ee/) (Table. S1). The RT-qPCR reactions were carried out on a LightCycler 480 System (Roche Diagnostics GmbH, Mannheim, Germany) using the first-strand cDNA templates, specific primers, and the SYBR Green PCR Master Mix (Thermo Fisher Scientific, Carlsbad, California). The thermocycling program consisted of an initial denaturation step at 95°C for 5 min, by 40 cycles of 95°C for 30 s 60°C for 30 s, and 72°C for 30 s, with an extension step at 72°C for 5 min for OPRM1, CCK, GCG and GABBR2, or by 40 cycles of 95°C for 30 s, 58 and 62°C for 30 s, and 72°C for 30 s, for KCND3 and TRH, respectively with an extension step at 72°C for 5 min. Relative gene expression was calculated using a comparative method ($2^{-\Delta\Delta CT}$) (Livak and Schmittgen, 2001) with β-actin as the internal control.

The expression values of the six genes from the RT-qPCR were transformed into log2 FC values and compared to those from RNA-Seq analysis. The correlation between expression values was calculated using the two-tailed unpaired Student's t-test (SPSS v. 24.0 for Windows software SPSS Inc., Chicago, IL).

4.4 Results and Discussion

4.4.1 Quality of RNA-Seq Reads

After removing low-quality and adaptor sequences, an average of 42,374,809 raw sequencing reads per sample were generated. The GC contents of the libraries ranged from 47 to 50%, which were very close to 50%. The Q20 and Q30 quality values were 96.87% and 92.13%, respectively in 10 libraries. The average ratio of high-quality reads to the reference genome was 83.62%. The detailed quality parameters of the RNA-seq reads are presented in Table 4.1.

Table 4.1 Number of RNA-Seq reads, quality and mapping rate of jejunum transcriptome of KR chickens between the control and the carnosine synthesis precursor groups.

Sample ID[1]	Raw Reads	Clean reads	Q20 (%)[2]	Q30 (%)[3]	GC content (%)	Total Mapped Read	Mapping Rage (%)
A1	41201870	40278408	96.99	92.40	48.52	36603341	85.25
A2	41353218	40193884	96.77	91.97	49.60	35576421	82.86
A3	43487456	42392812	96.91	92.22	49.80	38006681	83.90
A4	43402542	42257072	96.89	91.95	48.98	38363936	85.01
A5	40043532	38796770	96.67	91.86	49.53	34114019	81.36
B1	42917946	41821406	96.59	91.45	49.86	36678344	81.68
B2	43113272	42111470	96.91	92.22	49.13	37981503	84.45
B3	43249940	41903004	96.72	91.78	49.51	36858568	82.20
B4	42563958	41194424	96.96	92.36	49.22	37259521	84.77
B5	42414354	41140458	97.30	93.09	49.36	37158845	84.67
Average	42374808.8	41208970.8	96.87	92.13	49.35	36860117.9	83.62

[1]Sample ID represents treatment group and biological replicate number. Treatment groups are A (non-supplemented) and B (β-alanine and L-histidine supplementation). A1, A2, A3, A4, and A5 indicate the five biological replicate jejunal tissues in treatment group A, the rest of the groups (B) share the same name rules. [2] Q20 indicates the percentage of bases with a Q Phred value ≥ 20. [3] Q30 indicates the percentage of bases with a Q Phred value ≥ 30.

4.4.2 Differential Expression Genes Profiling

In total, 590 genes were expressed only in the control group, 341 genes were expressed only in the carnosine precursor group, and 13,372 genes were co-expressed in both libraries (Figure 4.1).

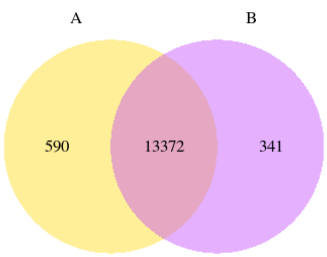

Figure 4.1 Venn diagram showing the number of genes expressed in jejunal tissues between the control (A) and the carnosine synthesis precursor (B) groups.

In total, 407 DEGs were identified; 135 genes were upregulated, and 272 genes were downregulated in the carnosine precursor group compared with the control group (Supplementary Table 2). The distinct transcriptional profiles between the control and the carnosine precursor groups are visualized with the volcano plots in Figure 4.2 and the clustering map of the DEGs showing the separation between the control and the carnosine precursor groups is given in Figure 4.3.

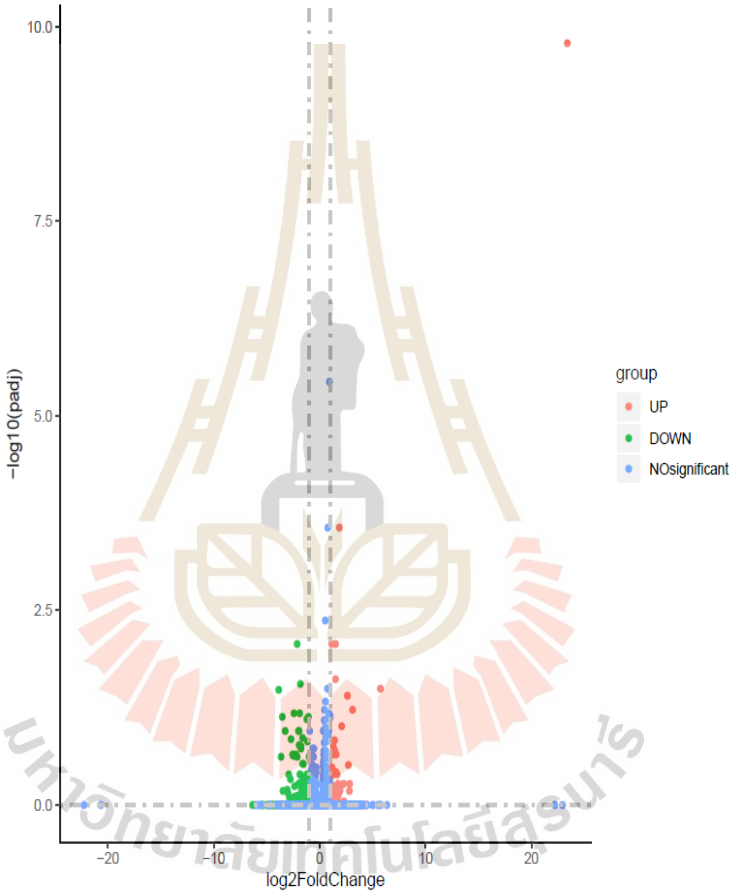

Figure 4.2 Volcano plot of differentially expressed genes in jejunal tissues between the control (A) and the carnosine synthesis precursor (B) groups. The horizontal lines indicate the significant thresholds of DEGs at a *Pue* < 0.05. The vertical line corresponds to the threshold of $|\log_2 FC| > 2$. Red dots represent the up-regulated genes in group B compared to group A, green down-regulated genes in group B compared to group A, and blue dots represent the genes that have similar expression in groups A and B.

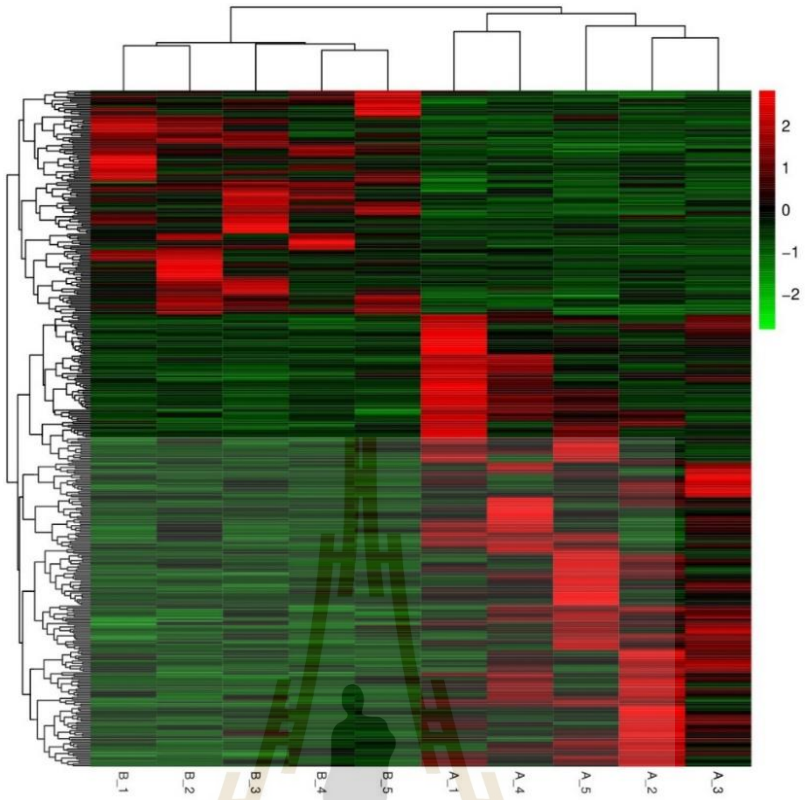

Figure 4.3 Hierarchical clustering of differentially expressed genes in jejunal tissues between the control (A) and the carnosine synthesis precursor (B) groups. Each row and column correspond to a differentially expressed gene or sample name, respectively. The red colors represent the overexpressed genes, and the green colors represent the genes with lower expression levels.

Most of the amino acids are absorbed in the jejunum by amino acid transporters and/or co-transporter (Broer, 2008). Our expectation of this result is to find the significance of DEGs related to absorption and transportation between groups with and without carnosine synthesis precursors. Our previous works (Kubota et al., 2021 and Suwanvichanee et al., 2022) indicated that β-alanine and/or L-histidine supplementation in diet enhanced carnosine synthesis in the KR breast muscle, increased carnosine content, improved meat quality and found significant differences between genes-related to meat tenderness in the breast meat of female KR when fed with β-alanine or L-histidine as dietary supplements. In the current study, we performed RNA-Seq to

identify DEGs and underlying molecular mechanisms related to β-alanine and L-histidine absorption and/or transportation in jejunum. Hence, GO annotation and KEGG pathway analysis will be contributed to better understand the different mechanistic pathways involved in the DEGs identified. The in-depth information and discussion will be described in the GO annotation and KEGG pathways analyses of DEGs.

4.4.3 GO Annotation and KEGG Pathways Analyses of DEGs

We conducted GO annotation analysis to associate the DEGs with their biological processes, molecular functions, and cellular components. Eighty-seven GO terms were significant ($P < 0.05$, $\log_2$ FC ≥ 2; Supplementary Table 3). The most significantly enriched GO terms are presented in Figure 4.4.

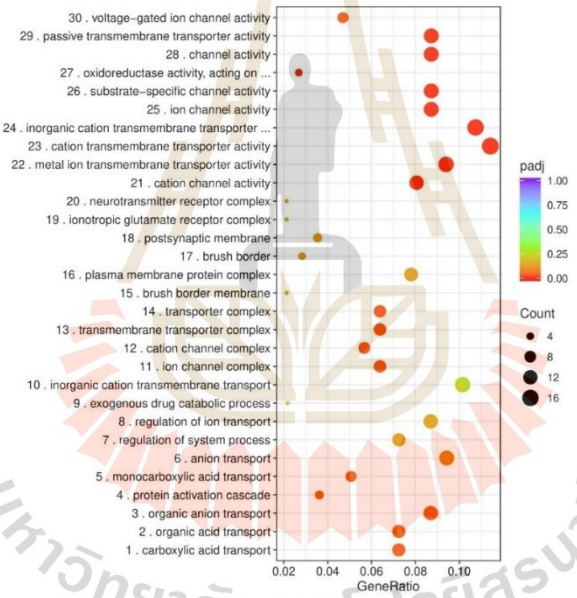

Figure 4.4 Top 30 enriched GO terms of DGEs in jejunum between the control (A) and the carnosine synthesis precursor (B) groups.

Additionally, KEGG pathway enrichment analysis showed that DEGs were significantly ($P < 0.05$, $\log_2$ FC ≥ 2) enriched in six signaling pathways: neuroactive ligand-receptor interaction, PPAR signaling pathway, linoleic acid metabolism, MAPK signaling pathway, calcium signaling pathway, and arachidonic acid metabolism. (Table 4.2).

Based on the GO terms, the main biological process implicated is carboxylic acid transport. The cellular component category indicated enrichment including transmembrane transporter complex, transporter complex, brush border membrane and plasma membrane protein complex. Additionally, in terms of molecular function indicates a significant difference in cation channel activity, ion channel activity, voltage-gated ion channel activity and metal ion transmembrane transporter activity.

Neuroactive ligand–receptor interaction pathway involves the interaction between various neuroactive molecules, including neurotransmitters (Squillario and Barla, 2011), hormones (Vaganova et al., 2023), and signaling molecules (Ji et al., 2018). This pathway related to amino acid absorption involves the transmission between neurotransmitters and receptors such as γ-Aminobutyric acid (GABA) in the gastrointestinal tract, which might regulate the absorption of amino acids (Dalangin et al., 2020). A previous study reported that β-alanine is a small molecule neurotransmitter giving that the structure of β-alanine intermediate between α-amino acid (glycine, glutamate) and GABA neurotransmitters (Tiedje et al., 2010). GABA is an inhibitory neurotransmitter synthesized from glutamate (Wang et al., 2023) and can act chelating ability of carnosine towards zinc (Zn^{2+}) and copper (Cu^{2+}) (Schön et al., 2019; Abate et al., 2022) as a co-transporter. Moreover, carnosine is a precursor to GABA, a key inhibitory neurotransmitter in the brain (Abate et al., 2022). Thus, carnosine precursor supplementation may affect the activity of neurotransmitter genes, hormones, and signaling molecules. Interestingly, genes involved in this pathway may also affect the absorption process by using neurotransmitter transporters that might increase the ability of absorption function in the jejunum

Table 4.2 Significantly enriched KEGG pathways in the jejunal tissues between the control and the β-alanine and L-histidine supplementation groups.

ID	Description	*P*-value	Gene Name
gga04080	Neuroactive ligand-receptor interaction	1.08E-05	SSTR2/P2RX1/GCG/GCGR/PTGER3/OPRM1/AGTR1/TRH/GRIN2C/GALR2/ GABBR2/CCK
gga03320	PPAR signaling pathway	1.15E-05	PLIN1/FABP4/FABP6/FABP3/HMGCS2/APOC3
gga00591	Linoleic acid metabolism	0.000496	CYP2C18
gga04010	MAPK signaling pathway	0.001495	AREG/CACNG3/IGF-I
gga04020	Calcium signaling pathway	0.003288	P2RX1/PTGER3/AGTR1/GRIN2C/TNNC2/NCX1
gga00590	Arachidonic acid metabolism	0.003521	CYP2C18

PPAR signaling pathway functions are associated with lipid and carbohydrate metabolism, energy balance, intestinal inflammation, hemostasis and ameliorating insulin sensitivity (Gharib-Naseri et al., 2021). Our study found, out of 63 DEGs, six are related to PPAR signaling pathway: Perilipin 1 (*PLIN1*), Fatty Acid-Binding Protein 4 (*FABP4*), Fatty Acid-Binding Protein 6 (*FABP6*), Fatty Acid-Binding Protein 3 (*FABP3)*, 3-Hydroxy-3-Methylglutaryl-CoA Synthase 2 (HMGCS2) and Apolipoprotein C3 (*APOC3*). These genes contribute to the absorption and transport of fatty acids in small intestine (Gaffar and Aathirah, 2023) and utilization of lipids (Ahmadian et al., 2013), which are essential processes for energy balance and metabolic health (Gharib-Naseri et al., 2021). Particularly, PPARα and PPARγ signaling can influence the expression of genes involved in amino acid transport and metabolism (Chen et al., 2015). A previous study demonstrated that activation of PPARα in intestine can enhance the expression of genes encoding amino acid transporters (B^0), thereby promoting the absorption of amino acids from the intestinal lumen into enterocytes (de Vogel-van den Bosch et al., 2008). PPAR signaling may also regulate the expression of genes involved in intracellular trafficking and vesicular transport mechanisms that facilitate the movement of amino acids across cellular channels (Glatz et al., 2010). Therefore, we might imply that the carnosine synthesis precursor as used as supplementation could activate the PPAR signaling pathway to increase the absorption of the amino acids by using the signaling transporters.

Moreover, among the significant KEGG pathways, our result found 2 pathways that are associated with fatty acid absorption including linoleic acid metabolism and arachidonic acid metabolism pathways. They are not directly linked with βA and L-His absorption and transportation in jejunum. However, a previous study reported that amino acid circulation imbalance is significantly associated with fatty acid metabolism disorder and enhanced oxidative stress (Jian et al., 2022). Wang et al. (2022) also reported that supplementation of amino acids may partly mediate the effect of dietary protein on the metabolism of protein, lipids and glucose, fertility, growth and production performance, and health of animals. Therefore, we might suggest that a supplement of carnosine precursor as an amino acid may also affect on fatty acid pathway in jejunum metabolic process.

In addition, MAPK signaling pathway plays a key role in innate immunity, inflammation, cell proliferation, cell differentiation, and cell death (Kim and Choi, 2015; Li and Wu, 2021). Three DEGs (Amphiregulin (*AREG*), Calcium Voltage-Gated Channel Auxiliary Subunit Gamma 3 (*CACNG3*) and Insulin-like growth factor I (*IGF-I*)) are functional in MAPK signaling pathway. Those genes are not directly connected with absorption or transportation in jejunum but could help to maintain intestinal epithelial barrier integrity and repair (Chen et al., 2018), improve mucosal structure and absorptive function (Zhang et al., 1995), and damage to the intestinal mucosal barrier (Liu et al., 2024). MAPK signaling is another important signaling pathway for amino acids and intestinal inflammation (He et al., 2018). Especially *CACNG3* gene that we found is related to implications for the modulation of smooth muscle contraction, neurotransmitter release, and cellular processes dependent on calcium signaling (Hofmann et al., 2014; Mochida, 2019). Therefore, the effect of amino acid carnosine supplementation may relate with voltage-gated channel activity, neurotransmitters and signals in the brush border membrane that are involved in absorption of carnosine precursor across the jejunum using a transporter.

Previous studies have reported that carnosine directly stimulates calcium ion (Ca^{2+}) release from the sarcoplasmic reticulum, increasing the Ca^{2+} sensitivity of ryanodine receptors and potentiating Ca^{2+} -induced Ca^{2+} release (Dutka et al., 2012). Thus, carnosine precursor supplementation that might relate to Calcium signaling pathway. Six DEGs relate to calcium signaling pathway: Purinergic Receptor P2X 1 (*P2RX1*), Prostaglandin E Receptor 3 (*PTGER3*), Angiotensin II Receptor Type 1 (*AGTR1*), glutamate ionotropic receptor NMDA type subunit 2C (*GRIN2C*), Troponin C2 (*TNNC2*), and Sodium/Calcium Exchanger 1 (*NCX1*). In small intestine, the absorptive route comprises the electrical entrance of Ca^{2+} by sodium-glucose co-transporter 1 (*SGLT-1*) and Na^{+}/K^{+}-ATPase. Two epithelial Ca^{2+} channels favor the passive transport of the cation across the apical membrane into enterocyte cytoplasm by transient receptor potential vanilloid 5 and 6 (*TRPV5* and *TRPV6*) (Areco et al., 2020). Even though, the connection between the calcium signaling pathway and the absorption and transportation of β-alanine and L-histidine in jejunum is unclear, Calcium signaling pathway is very interesting and further studies are needed to examine the role of the

calcium signaling pathway and the regulated genes in receptors and exchangers in jejunum.

4.4.4 Protein-protein interaction (PPI) network analysis of DEGs

PPI network analysis showed that the pathways involving *KCND3, OPRM1, CCK, GCG, TRH,* and *GABBR2* were in the core nodes of the PPI network, these genes enriched in neuroactive ligand-receptor interaction pathway (Figure 4.5) and might influence β-alanine and L-histidine transportation in jejunum.

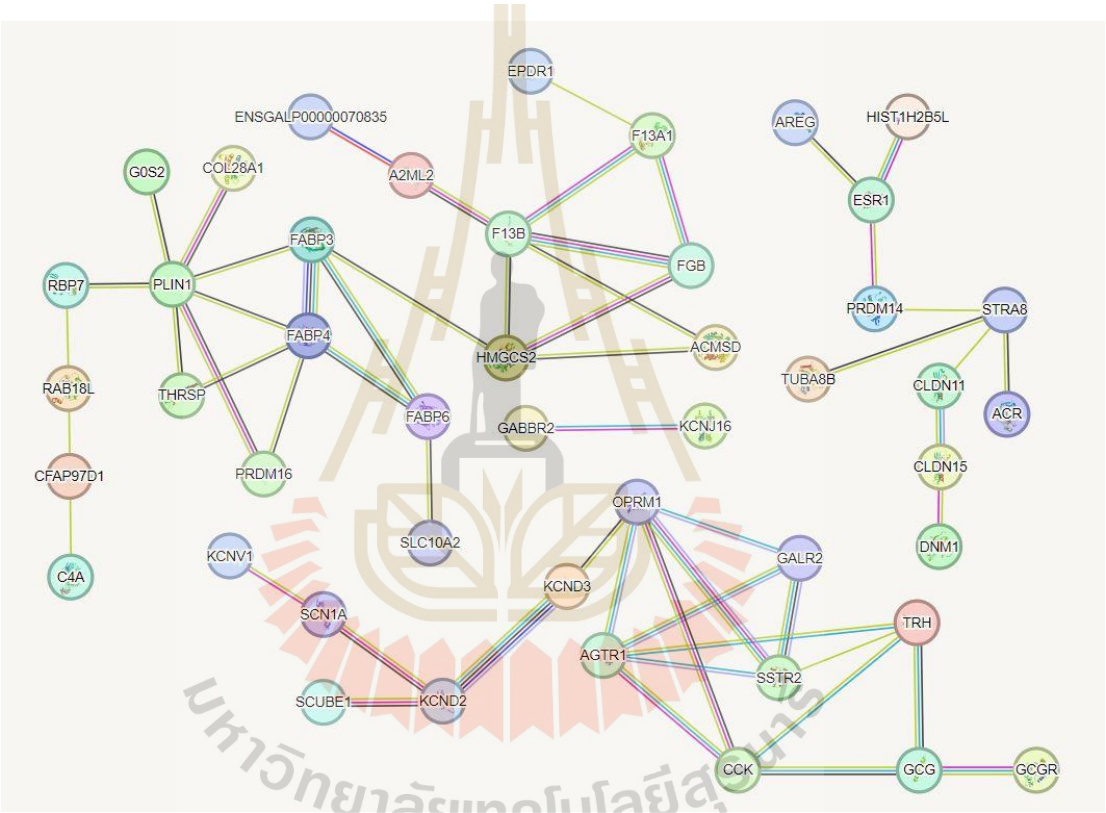

Figure 4.5 Protein-protein interaction network of DEGs in jejunum.

We found the ligand–receptor interaction related genes, *KCND3, OPRM1, CCK*, and *GCG* were down-regulate in the experimental group compared to control group. Potassium voltage-gated channel subfamily D member 3 (*KCND3)* encodes a member of the potassium voltage-gated channel, that are related to peptide transporter 1 (***PEPT1)*** in enterocytes of the small intestine across apical membrane (Wu, 2020). The absorption of β-alanine and/or L-his by *PEPT1* is accompanied by the

translocation of proton and thus positive charge movement (Viennois et al., 2018). The sodium-potassium (**Na^+-K^+**) -ATPase (sodium co-transport), localized in the basolateral membrane of enterocytes, plays a major role in nutrient transport in the small intestine by transferring K^+ ions into and Na^+ out of the cell (Gal-Garber et al., 2003). Because carnosine can be transported into cells by *PEPT1*, and hydrolysis as K+ ions activity in the intracellular, then amino acids are released into the cell via basolateral amino acid transporters (Viennois et al., 2018). Therefore, we might assume that the supplement the carnosine synthesis precursors could be linked to *KCND3* to absorb β-alanine and L-histidine into the muscle by sodium co-transport for carnosine synthesis.

The other interesting down-regulated gene in the group fed with carnosine precursors was opioid receptor mu 1 (*OPRM1). OPRM1* is associated with norepinephrine and lactate changes in blood (Karoly et al., 2012; Toddes et al., 2021) and inhibits neurotransmitter release by reducing Ca^{2+} currents (Al-Hasani and Bruchas, 2011). Our previous study also found that β-alanine and L-histidine supplementation affected lactate levels in the chicken jejunum (Promkhun et al., 2023). This could be due to low expression of *OPRM1* found in chicken fed with the carnosine precursors. Therefore, low expression of *OPRM1* may be the key involved in the absorption by transport of carnosine precursors in the chicken intestine related to Ca^{2+} ion channel.

Another gene with low expression in the experimental group, was cholecystokinin (*CCK*), which is generally considered as a satiety hormone in poultry (Reid and Dunn, 2018). Transcripts of *CCK* in the gastrointestinal tract are dispersed throughout the small intestine (Wölnerhanssen et al., 2017). Digested proteins, fats and aromatic amino acids are major stimulants of *CCK* release (Wang et al., 2011). However, the cellular mechanism by which amino acids affect *CCK* secretion is unknown. In our previous study, we found that diet supplemented with β-alanine and L-histidine increases the amount of phenylalanine in the jejunum (Promkhun et al., 2023). Thus, phenylalanine has a direct effect on CCK intestinal cells by activating the Ca^{2+}-sensing receptor (CaSR) that stimulates CCK release (Wang et al., 2011). In addition to that, administration of phenylalanine inhibits K^+ channel activity and causes CCK cell alteration (Schwaiger et al., 2013), consistent with changes necessary for hormone

secretion (Wang et al., 2011). This may indicate that *CCK* might affect K^+ channel activity in jejunum which might be related to carnosine precursor supplementation absorption.

In addition, Gallus gallus thyrotropin-releasing hormone gene (*TRH)* was up-regulated in in the experimental group compared to control group. TRH is a major hypothalamic factor in the regulation of thyrotropin secretion (Nillni, 2010). The physiological roles of TRH are mediated by thyrotropin-releasing hormone receptor genes (***TRHR***) that belong to G protein–coupled receptor gene family (Creighton et al., 2022). TRH is characterized by substitution of basic amino acid histidine (related to authentic TRH) with neutral or acidic amino acids (Pekary et al. 1983). TRH can be found in the hypothalamus, and in prostate gland, male reproductive system, retina, placenta, brain and certain endocrine tissues, and gastrointestinal tract (Bilek, 2000). Creighton et al. (2022) indicated that *TRHR* can couple to G protein upon ligand activation and this activation can stimulate intracellular Ca^{2+} mobilization. The muscle Ca^{2+} release channels contain saturable binding sites for carnosine, indicating that carnosine has the potential to alter the Ca^{2+} channel itself (Jones et al., 2017). Therefore, the β-alanine and L-histidine supplementing may stimulate the increase of Ca^{2+} release and accumulation of Ca^{2+} in the muscles because our previous study found the high carnosine content in breast meat (Suwanvichanee et al., 2022) this may indicate that Ca^{2+} might relate to the ability of transportation of β-alanine and L-histidine in jejunum.

Moreover, gamma-aminobutyric acid type B receptor subunit 2 (*GABBR2)* gene was up-regulated in the experimental group compared to control group. *GABBR2* belongs to the GABA-B receptors that inhibit neuronal activity through G protein-coupled second-messenger systems; which regulates the release of neurotransmitters, and the activity of ion channels and adenylyl cyclase (Bettler et al., 2004). Supplementing diet with the carnosine precursors β-alanine and L-histidine, results in decline in muscle half-relaxation time potentially via alterations in Ca^{2+} handling (Everaert et al., 2013). Given that accumulation of hydrogen cation (H^+) impacts Ca^{2+} signaling during muscular contraction, carnosine has a potential to serve as a cytoplasmic regulator for Ca^{2+} and H^+ because both ions bind together (Jones et al., 2017). Consequently, supplementation of carnosine precursors in diet may affect the

activation of Ca^{2+} channels and higher expression of *GABBR2* in the experimental group compared to control group.

We did not find major genes involved in absorption and transportation of carnosine precursors, β-alanine and L-histidine in jejunum. Nevertheless, the identified DEGs may indicate the presence of genes potentially serving as neurotransmitter, receptors, or signaling molecules that are related to transport activity or channel function across the jejunum enterocyte cell. Therefore, it is interesting to note that in addition to genes that play a role in absorption and transportation, there are other groups of genes that can be expressed differently by carnosine precursor supplementation.

4.4.5 Validation of DEGs by RT-PCR

The six DEGs (*TRH, GABBR2, KCND3, OPRM1, CCK,* and *GCG*) that were highly connected in PPI network analysis and neuroactive ligand–receptor interaction pathway were selected for verification. Expression patterns of these genes between the RT-qPCR and the RNA-seq methods had a strong positive correlation of r=0.91 ($P < 0.05$) (Figure 4.6). Thus, the RNA-Seq results in this study can be considered as accurate and reproducible.

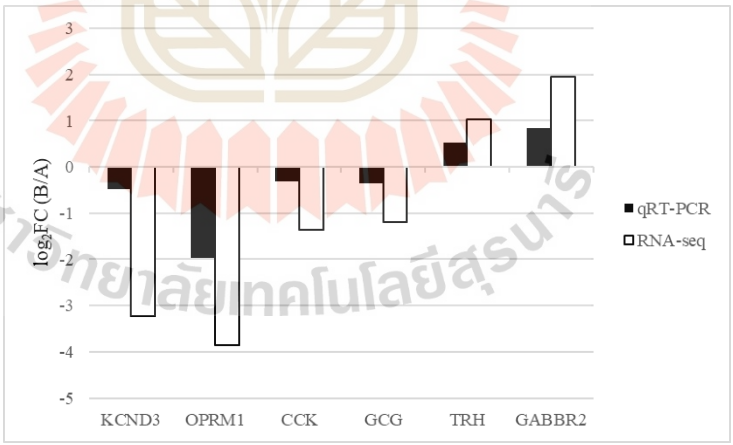

Figure 4.6 Expression of six random DGEs detected using either RNA-seq or RT-qPCR. Abbreviations: A, the control group; B, the carnosine synthesis precursor group, Gene id; full name, potassium voltage-gated channel subfamily D member 1 (*KCND3*), opioid receptor mu 1 (*OPRM1*), cholecystokinin (*CCK*), glucagon (*GCG*), thyrotropin-releasing hormone (*TRH*), and gamma-aminobutyric acid type B receptor subunit 2 (*GABBR2*).

4.5 Conclusion

This is the first report on global gene expression in chicken jejunum related to β-alanine and L-histidine absorption and transportation. A total of 272 genes, down-regulated and 135 up-regulated were identified in chicken jejunum in a group fed with or without carnosine precursors β-alanine and L-histidine supplementation. Our finding can imply the genes that can be used as a neurotransmitter, receptors, or signaling molecules that related to transportation activity using ion channel function across the jejunum enterocyte cell. The pathway enrichment analysis revealed that the main biological pathways related to DEGs were related to neuroactive ligand–receptor interaction, MAPK signaling pathway, PPAR signaling pathway, and Calcium signaling pathway. These findings advance our understanding of the molecular mechanism and genes related to the absorption and transportation of β-alanine and/or L-histidine in chicken jejunum and can also be used as a gene marker or biomarker to improve carnosine content in slow-growing KR chicken. However, it is important to note that the specific mechanisms of carnosine synthesis, absorption, transportation, and utilization in chickens are still an active area of research, and more studies are needed to fully understand the role of carnosine synthesis in chickens.

4.6 References

Abate, C., Aiello, D., Cordaro, M., Giuffrè, O., Napoli, A., and Foti, C. (2022). Binding ability of L-carnosine towards Cu2+, Mn2+ and Zn2+ in aqueous solution. **Journal of Molecular Liquids**. 368. 120772.

Ahmadian, M., Suh, J. M., Hah, N., Liddle, C., Atkins, A. R., Downes, M., and Evans, R. M. (2013). PPARγ signaling and metabolism: the good, the bad and the future. **Nature medicine**. 19(5). 557-566.

Al-Hasani, R., and Bruchas, M. R. (2011). Molecular mechanisms of opioid receptor-dependent signaling and behavior. **The Journal of the American Society of Anesthesiologists**. 115(6). 1363-1381.

Areco, V. A., Kohan, R., Talamoni, G., de Talamoni, N. G. T., and López, M. E. P. (2020). Intestinal Ca2+ absorption revisited: A molecular and clinical approach. **World Journal of Gastroenterology**. 26(24). 3344.

Bauchart, C., Savary-Auzeloux, I., Patureau Mirand, P., Thomas, E., Morzel, M., and Rémond, D. (2007). Carnosine concentration of ingested meat affects carnosine net release into the portal vein of minipigs. **The Journal of nutrition**. 137(3). 589-593.

Bettler, B., Kaupmann, K., Mosbacher, J., and Gassmann, M. (2004). Molecular structure and physiological functions of GABAB receptors. **Physiological reviews**. 84(3). 835-867.

Bilek, R. (2000). TRH-like peptides in prostate gland and other tissues. **Physiological Research**. 49: S19-S26.

Blancquaert, L., Baba, S. P., Kwiatkowski, S., Stautemas, J., Stegen, S., Barbaresi, S., and Delanghe, J. (2016). Carnosine and anserine homeostasis in skeletal muscle and heart is controlled by β-alanine transamination. **The Journal of physiology**. 594(17). 4849-4863.

Bonfanti, L., Peretto, P., De Marchis, S., and Fasolo, A. (1999). Carnosine-related dipeptides in the mammalian brain. **Progress in neurobiology**. 59(4). 333-353.

Brosnan, M. E., and Brosnan, J.T. (2020). Histidine metabolism and function. **The Journal of Nutrition**. 150(Supplement_1). 2570S-2575S.

Cararo, J. H., Streck, E. L., Schuck, P. F., and da C Ferreira, G. (2015). Carnosine and related peptides: therapeutic potential in age-related disorders. **Aging and disease**. 6(5). 369.

Chen, Z., He, P., Ding, X., Huang, Y., Gu, H., and Ni, X. (2015). PPARγ stimulates expression of L-type amino acid and taurine transporters in human placentas: the evidence of PPARγ regulating fetal growth. **Scientific Reports**. 5(1). 12650.

Chen, F., Yang, W., Huang, X., Cao, A. T., Bilotta, A. J., Xiao, Y., and Cong, Y. (2018). Neutrophils promote amphiregulin production in intestinal epithelial cells through TGF-β and contribute to intestinal homeostasis. **The Journal of Immunology**. 201(8). 2492-2501.

Creighton, J. V., de Souza Gonçalves, L., Artioli, G. G., Tan, D., Elliott-Sale, K. J., Turner, M. D., and Sale, C. (2022). Physiological Roles of Carnosine in Myocardial Function and Health. **Advances in Nutrition**. 13(5). 1914-1929.

Dalangin, R., Kim, A., and Campbell, R. E. (2020). The role of amino acids in neurotransmission and fluorescent tools for their detection. **International journal of molecular sciences**. 21(17). 6197.

de Vogel-van den Bosch, H. M., Bünger, M., de Groot, P. J., Bosch-Vermeulen, H., Hooiveld, G. J., and Müller, M. (2008). PPARalpha-mediated effects of dietary lipids on intestinal barrier gene expression. **BMC genomics**. 9. 1-13.

Dutka, T. L., Lamboley, C. R., McKenna, M. J., Murphy, R. M., and Lamb, G. D. (2012). Effects of carnosine on contractile apparatus Ca2+ sensitivity and sarcoplasmic reticulum Ca2+ release in human skeletal muscle fibers. **Journal of applied physiology**. 112(5). 728-736.

Everaert, I., Stegen, S., Vanheel, B., Taes, Y., and W. Derave. (2013). Effect of beta-alanine and carnosine supplementation on muscle contractility in mice. **Medicine and science in sports and exercise**. 45(1). 43-51.

Ferraris, R. P., Diamond, J. A. R. E. D., and Kwan, W. W. (1988). Dietary regulation of intestinal transport of the dipeptide carnosine. **American Journal of Physiology-Gastrointestinal and Liver Physiology**. 255(2). G143-G150.

Franceschini, A., Szklarczyk, D., Frankild, S., Kuhn, M., Simonovic, M., Roth, A., and Jensen, L. J. (2012). STRING v9. 1: protein-protein interaction networks, with increased coverage and integration. **Nucleic acids research**. 41(D1). D808-D815.

Gal-Garber, O., Mabjeesh, S. J., Sklan, D., and Uni, Z. (2003). Nutrient transport in the small intestine: Na+, K+-ATPase expression and activity in the small intestine of the chicken as influenced by dietary sodium. **Poultry Science**. x 82(7). 1127-1133.

Gharib-Naseri, K., de Las Heras-Saldana, S., Kheravii, S., Qin, L., Wang, J., and Wu, S. B. (2021). Necrotic enteritis challenge regulates peroxisome proliferator-1 activated receptors signaling and β-oxidation pathways in broiler chickens. **Animal Nutrition**. 7(1). 239-251.

Glatz, J. F., Luiken, J. J., and Bonen, A. (2010). Membrane fatty acid transporters as regulators of lipid metabolism: implications for metabolic disease. **Physiological reviews**. 90, 367–417.

He, F., Wu, C., Li, P., Li, N., Zhang, D., Zhu, Q., and Peng, Y. (2018). Functions and signaling pathways of amino acids in intestinal inflammation. **BioMed research international**. 2018.

Hofmann, F., Flockerzi, V., Kahl, S., and Wegener, J. W. (2014). L-type CaV1. 2 calcium channels: from in vitro findings to in vivo function. **Physiological reviews**. 94(1). 303-326.

Intarapichet, K. O., and Maikhunthod, B. (2005). Genotype and gender differences in carnosine extracts and antioxidant activities of chicken breast and thigh meats. **Meat Science**. 71(4). 634-642.

Ji, X., Bossé, Y., Landi, M. T., Gui, J., Xiao, X., Qian, D., and Amos, C.I. (2018). Identification of susceptibility pathways for the role of chromosome 15q25. 1 in modifying lung cancer risk. **Nature Communications**. 9(1). 3221.

Jian, H., Xu, Q., Wang, X., Liu, Y., Miao, S., Li, Y., and Zou, X. (2022). Amino acid and fatty acid metabolism disorders trigger oxidative stress and inflammatory response in excessive dietary valine-induced NAFLD of laying hens. **Frontiers in nutrition**. 9. 849767.

Jones, R. L., Barnett, C. T., Davidson, J., Maritza, B., Fraser, W. D., Harris, R., and Sale, C. (2017). β-alanine supplementation improves in-vivo fresh and fatigued skeletal muscle relaxation speed. **European Journal of Applied Physiology**. 117. 867-879.

Jukić, I., Kolobarić, N., Stupin, A., Matić, A., Kozina, N., Mihaljević, Z., and Drenjančević, I. (2021). Carnosine, small but mighty—Prospect of use as functional ingredient for functional food formulation. **Antioxidants**. 10(7). 1037.

Karoly, H. C., Stevens, C. J., Magnan, R. E., Harlaar, N., Hutchison, K. E., and Bryan, A. D. (2012). Genetic influences on physiological and subjective responses to an aerobic exercise session among sedentary adults. **Journal of cancer epidemiology**. 2012.

Katemala, S., Molee, A., Thumanu, K., and Yongsawatdigul, J. (2021). Meat quality and Raman spectroscopic characterization of Korat hybrid chicken obtained from various rearing periods. **Poultry Science**. 100(2). 1248-1261.

Kai, S., Watanabe, G., Kubota, M., Kadowaki, M., and Fujimura, S. (2015). Effect of dietary histidine on contents of carnosine and anserine in muscles of broilers. **Animal Science Journal**. 86(5). 541-546.

Kim, E. K., and Choi, E. J. (2015). Compromised MAPK signaling in human diseases: an update. **Archives of toxicology**. 89. 867-882.

Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., and Salzberg, S.L. (2013). TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. **Genome biology**. 14(4). 1-13.

Kopec, W., Jamroz, D., Wiliczkiewicz, A., Biazik, E., Pudlo, A., Korzeniowska, M., and Skiba, T. (2020). Antioxidative characteristics of chicken breast meat and blood after diet supplementation with carnosine, L-histidine, and β-alanine. **Antioxidant**. 9(11). 1093.

Kralik, G., Sak-Bosnar, M., Kralik, Z., Galović, O., Grčević, M., and Kralik, I. (2015). Effect of β-alanine and L-histidine on concentration of carnosine in muscle tissue and oxidative stability of chicken meat. **PoljoPrivreda**. 21(1 SUPPLEMENT). 190-194.

Kubota, S., Promkhun, K., Sinpru, P., Suwanvichanee, C., Molee, W., and Molee, A. (2021). RNA profiles of the korat chicken breast muscle with increased carnosine content produced through dietary supplementation with β-alanine or L-histidine. **Animals**. 11(9). 2596.

Lackner, J., Albrecht, A., Mittler, M., Marx, A., Kreyenschmidt, J., Hess, V., and Sauerwein, H. (2021). Effect of feeding histidine and β-alanine on carnosine concentration, growth performance, and meat quality of broiler chickens. **Poultry Science**. 100(11). 101393.

Li, D., and Wu, M. (2021). Pattern recognition receptors in health and diseases. **Signal transduction and targeted therapy**. 6(1). 291.

Liu, L., Gong, X., Zhang, X., Zhang, D., Tang, Y., Liu, J., and Pan, D. (2024). Resveratrol alleviates heat-stress-induced impairment of the jejunal mucosa through TLR4/MAPK signaling pathway in black-boned chicken. **Poultry Science**. 103(1). 103242.

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2– ΔΔCT method. **Methods**. 25(4). 402-408.

Maikhunthod, B. and Intarapichet, K.O. (2005). Heat and ultrafiltration extraction of broiler meat carnosine and its antioxidant activity. **Meat Science**. 71. 364-374.

Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. **EMBnet. Journal**. 17(1):10-12.

Miyaji, T., Sato, M., Maemura, H., Takahata, Y., and Morimatsu, F. (2012). Expression profiles of carnosine synthesis–related genes in mice after ingestion of carnosine or β-alanine. **Journal of the International Society of Sports Nutrition**. 9(1). 15.

Mochida, S. (2019). Presynaptic calcium channels. **International Journal of Molecular Sciences**. 20(9). 2217.

Nillni, E. A. (2010). Regulation of the hypothalamic thyrotropin releasing hormone (TRH) neuron by neuronal and peripheral inputs. **Frontiers in neuroendocrinology**. 31(2). 134-156.

Pekary, A. E., Ross, R., Dekernion, J., & Hershman, J. M. (1983). Human reproductive tissues contain thyrotropin releasing hormone (TRH) and TRH-homologous peptides. **Journal of andrology**. 4(6). 408-414.

Promkhun, K., Suwanvichanee, C., Tanpol, N., Katemala, S., Thumanu, K., Molee, W., and Molee, A. (2023). Effect of carnosine synthesis precursors in the diet on jejunal metabolomic profiling and biochemical compounds in slow-growing Korat chicken. **Poultry Science**. 102(12). 103123.

Reid, A. M., and Dunn, I. C. (2018). Gastrointestinal distribution of chicken gastrin-cholecystokinin family transcript expression and response to short-term nutritive state. **General and comparative endocrinology**. 255. 64-70.

Sadikali, F., Darwish, R., and Watson, W. C. (1975). Carnosinase activity of human gastrointestinal mucosa. **Gut**. 16(8). 585-589.

Sale, C., Artioli, G. G., Gualano, B., Saunders, B., Hobson, R. M., and Harris, R. C. (2013). Carnosine: from exercise performance to health. **Amino acids**. 44. 1477-1491.

Schön, M., Mousa, A., Berk, M., Chia, W. L., Ukropec, J., Majid, A., and De Courten, B. (2019). The potential of carnosine in brain-related disorders: A comprehensive review of current evidence. **Nutrients**. 11(6). 1196.

Schwaiger, C. S., Liin, S. I., Elinder, F., and E. Lindahl. 2013. The conserved phenylalanine in the K+ channel voltage-sensor domain creates a barrier with unidirectional effects. **Biophysical journal**. 104(1). 75-84.

Sinpru, P., Riou, C., Kubota, S., Poompramun, C., Molee, W., and Molee, A. (2021). Jejunal transcriptomic profiling for differences in feed conversion ratio in slow-growing chickens. **Animals**. 11(9). 2606.

Stenesh, J. J., and Winnick, T. (1960). Carnosine–anserine synthetase of muscle. 4. Partial purification of the enzyme and further studies of β-alanyl peptide synthesis. **Biochemical Journal**, 77(3), 575.

Suwanvichanee, C., Sinpru, P., Promkhun, K., Kubota, S., Riou, C., Molee, W., and Molee, A. (2022). Effects of β-alanine and L-histidine supplementation on carnosine contents in and quality and secondary structure of proteins in slow-growing Korat chicken meat. **Poultry Science**. 101(5). 101776.

Squillario, M., and Barla, A. (2011). A computational procedure for functional characterization of potential marker genes from molecular data: Alzheimer's as a case study. **BMC medical genomics**. 4. 1-16.

Tiedje, K. E., Stevens, K., Barnes, S., and Weaver, D. F. (2010). β-Alanine as a small molecule neurotransmitter. **Neurochemistry international**. 57(3). 177-188.

Toddes, C., Lefevre, E. M., Brandner, D. D., Zugschwert, L., and Rothwell, P. E. (2021). μ-Opioid receptor (Oprm1) copy number influences nucleus accumbens microcircuitry and reciprocal social behaviors. **Journal of Neuroscience**. 41(38). 7965-7977.

Tomonaga, S., Matsumoto, M., and Furuse, M. (2012). β-Alanine enhances brain and muscle carnosine levels in broiler chicks. **The Journal of Poultry Science**. 49(4). 308-312.

Trapnell, C., Williams, B. A., Pertea, G., Mortazavi, A., Kwan, G., Van Baren, M. J., and Pachter, L. (2010). Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. **Nature biotechnology**. 28(5). 511-515.

Vaganova, A. N., Maslennikova, D. D., Konstantinova, V. V., Kanov, E. V., and Gainetdinov, R.R. (2023). The Expression of Trace Amine-Associated Receptors (TAARs) in Breast Cancer Is Coincident with the Expression of Neuroactive Ligand–Receptor Systems and Depends on Tumor Intrinsic Subtype. **Biomolecules.** 13(9). 1361.

Viennois, E., Pujada, A., Zen, J., and Merlin, D. (2018). Function, regulation, and pathophysiological relevance of the POT superfamily, specifically PepT1 in inflammatory bowel disease. **Comprehensive Physiology**. 8(2). 731.

Wang, Y., Chandra, R., Samsa, L. A., Gooch, B., Fee, B. E., Cook, J. M., and Liddle, R. A. (2011). Amino acids stimulate cholecystokinin release through the Ca^{2+}-sensing receptor. **American Journal of Physiology-Gastrointestinal and Liver Physiology**. 300(4). G528-G537.

Wang, S., Jung, S., and Ko, K. S. (2022). Effects of amino acids supplementation on lipid and glucose metabolism in HepG2 cells. **Nutrients**. 14(15). 3050.

Wang, X., Su, Y., Cai, Z., Xu, Y., Wu, X., Hua, C., and Zhou, Q. (2023). **γ**-Aminobutyric acid promotes the inhibition of hair growth induced by chronic restraint stress. **Life Sciences.** 317. 121439.

Wölnerhanssen, B. K., Moran, A. W., Burdyga, G., Meyer-Gerspach, A. C., Peterli, R., Manz, M., and Shirazi-Beechey, S. P. (2017). Deregulation of transcription factors controlling intestinal epithelial cell differentiation; a predisposing factor for reduced enteroendocrine cell number in morbidly obese individuals. **Scientific reports**. 7(1). 8174.

Wu, G. (2020). Important roles of dietary taurine, creatine, carnosine, anserine and 4-hydroxyproline in human nutrition and health. **Amino acids**. 52(3). 329-360.

Xing, L., Chee, M. E., Zhang, H., Zhang, W., and Mine, Y. (2019). Carnosine—A natural bioactive dipeptide: Bioaccessibility, bioavailability and health benefits. **Journal of Food Bioactives**. 5. 8-17.

Zhang, W., Frankel, W. L., Adamson, W. T., Roth, J. A., Mantell, M. P., Bain, A., and Rombeau, J. L. (1995). Insulin-like growth factor-I improves mucosal structure and function in transplanted rat small intestine. **Transplantation**. 59(5). 755-761.

CHAPTER V

SUMMARY

Our research can conclude that metabolites may play important roles in the overall metabolism involved in carnosine synthesis and may be associated with precursor absorption and transportation in KR jejunum especially β-alanine, butyrate, choline, creatine, glutamate, glutamine, lactate, methionine, myo-inositol, betaine, threonine, and taurine. Furthermore, 34 metabolic pathways showed differentiation between the groups. We expected to identify carnosine synthesis-related metabolic pathways, especially β-alanine and L-histidine metabolisms, as the main metabolic pathways affected by β-alanine and L-histidine supplementation. Even though the differences between the control and the supplementation groups were not statistically significant but had high impact values. In addition, we found other pathways involved in β-alanine, L-histidine, and carnosine metabolism, e.g., alanine metabolism.

The relative proportion of different protein secondary structures indicated that the β-alanine precursor in the diet increases β-sheets and decreases α-helix in the amide I region in jejunal tissue. Moreover, an L-histidine precursor in the diet increases β-sheets in the amide I region. Principal component analysis revealed that a diet with both precursors of the carnosine synthesis is strongly and positively associated with amide I, amide II, creatine, tyrosine, valine, isoleucine, and aspartate levels in the jejunum. Therefore, our study can confirm that precursor supplements in the diet influence biochemical changes in jejunal tissue and can be used for monitoring the precursor response.

To study gene expression in jejunal tissue under a diet with and without carnosine synthesis precursors, we found 6 genes including *KCND3, OPRM1, CCK, GCG, TRH,* and *GABBR2* that might relate to gene expression of carnosine-related enzymes and transporters. The pathway enrichment analysis revealed that the main biological pathways involved in jejunal differences in the group with carnosine synthesis

precursors were those related to neuroactive ligand–receptor interaction, MAPK signaling pathway, PPAR signaling pathway, and calcium signaling pathway. These findings can serve to explore the molecular mechanism and genes related to β-alanine and L-histidine absorption and/or transportation of the jejunum function and may help to identify molecular markers that can be used for slow-growing KR chicken genetic selection to improve carnosine content in KR chicken meat. However, it is important to note that the specific mechanisms of carnosine synthesis, transportation, and utilization in chickens are still an active area of research, and more studies are needed to fully understand the role of carnosine in chickens. Moreover, these findings provide the confidence to step forward for genetic improvement and selection to enhance carnosine synthesis ability without a negative impact on the molecular function in chickens.

APPENDIX

QR code for Supplementary file

Table S1. List of primer sequences used for quantitative real-time PCR validation.

Table S2. List of all differentially expressed genes in jejunal tissue between control group (A) and carnosine synthesis precursors (B) group.

Table S3. List of GO terms for differentially expressed genes in jejunal tissue between control group and carnosine synthesis precursors group.

Promkhun, K., Suwanvichanee, C., Tanpol, N., Katemala, S., Thumanu, K., Molee, W., and Molee, A. (2023). Effect of carnosine synthesis precursors in the diet on jejunal metabolomic profiling and biochemical compounds in slow-growing Korat chicken. **Poultry Science**. 102(12):103123.

BIOGRAPHY

Kasarat Satukangas (Promkhun) was born on November 28, 1994, at Surin, Thailand. In 2013, finished high school at Sirindhorn School, Surin. In 2017, graduated with a bachelor's degree (first honors) from the School of Animal Technology and Innovation at Suranaree University of Technology (SUT), Nakhon Ratchasima. Then, she began her Ph.D. program in the School of Animal Technology and Innovation, Institute of Agricultural Technology, SUT, Nakhon Ratchasima, as she received a scholarship from Thailand Research Fund (TRF) and Synchrotron Light Research Institute (Public Organization) for their joint financial support through the Royal Golden Jubilee Ph.D. (RGJ-PHD) Program (Grant No. PHD/0196/2559) 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 an exchange student, learning about quantitative genetic, population genetic, gene mapping and transcriptomic bioinformatics.

She presented poster presentations including: 1) International Symposium Nakhon Ratchasima, Thailand. 2) The 7th SUT International Colloquium on Agricultural Technology, Nakhon Ratchasima, Thailand. 3) The 39th International Society for Animal Genetics Conference, Cape Town, South Africa.

She publicized including: 1) Promkhun**, K.**, Suwanvichanee, C., Tanpol, N., Katemala, S., Thumanu, K., Molee, W., and Molee, A. (2023). Effect of carnosine synthesis precursors in the diet on jejunal metabolomic profiling and biochemical compounds in slow-growing Korat chicken. Poultry Science, 102(12), 103123. 2) Suwanvichanee, C., P. Sinpru, **K. Promkhun**, S. Kubota, C. Riou, W. Molee, J. Yongsawatdigul, K. Thumanu, and A. Molee. 2022. Effects of β-alanine and L-histidine supplementation on carnosine contents in and quality and secondary structure of proteins in slow-growing Korat chicken meat. Poultry Science:101776. **And 3)** Kubota, S., **K. Promkhun**, P. Sinpru, C. Suwanvichanee, W. Molee, and A. Molee. 2021. RNA Profiles of the Korat Chicken Breast Muscle with Increased Carnosine Content Produced through Dietary Supplementation with β-Alanine or L-Histidine. Animals 11:2596.