DETERMINING THE ROLE OF TRYPTOPHAN, ISOLEUCINE AND VALINE IN REGULATION OF GROWTH AND NUTRIENT METABOLISM IN YOUNG PIGS

By

PARNIYAN GOODARZI

Bachelor of Science in Animal Science
Shiraz University
Shiraz, Fars
2015

Master of Science in Animal Physiology
Shiraz University
Shiraz, Fars
2017

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 2022

DETERMINING THE ROLE OF TRYPTOPHAN, ISOLEUCINE AND VALINE IN REGULATION OF GROWTH AND NUTRIENT METABOLISM IN YOUNG PIGS

Dissertation Approved:

Dr. Adel Pezeshki

Dissertation Adviser

Dr. Scott D. Carter

Dr. Glenn Zhang

Dr. Dingbo Lin

.

ACKNOWLEDGEMENTS

Ph.D. is a journey with ups and downs, but it will be easier with people who support, motivate, and encourage you. My advisor, Dr. Adel Pezeshki, is a person that I would like to express my sincere gratitude and appreciation for his guidance toward the last year of my Ph.D. His support, inspiration and encouragement lead to my achievements during my Ph.D. My sincere thanks go to my committee members, Dr. Scott Carter, Dr. Glenn Zhang and Dr. Dingbo (Daniel) Lin, for their advice and assistance.

I would like to thank my lab mates and undergrad students (Mohammad Habibi, Cedrick Shili, Kennedy Roberts, Julia Sutton, Mathew Gorton, Katy Walsh) who helped me during my doctoral studies and for all the fun we had at Oklahoma State University.

I cannot begin to express my thanks to the Department of Animal and Food Sciences family at Oklahoma State University, wonderful faculty members, staff, and graduate students, who warmly embraced us to make Stillwater feel like home.

I would like to thank my parents who always encourage and motivate me for my future goals. I am proud to have such parents. Thank you to my sisters who are my best friends and always support me and have my best interest at heart.

My sincere thanks goes to my husband, Mohammad Habibi, who supported me during this journey and gave me inspiration with his warm heart.

Last but not the least, I am very proud of my brave people in **Iran** especially courageous women who fight for their basic rights and liberty. **Woman, Life, Freedom!**

Name: PARNIYAN GOODARZI

Date of Degree: DECEMBER, 2022

Title of Study: DETERMINING THE ROLE OF TRYPTOPHAN, ISOLEUCINE AND VALINE IN REGULATION OF GROWTH AND NUTRIENT METABOLISM IN YOUNG PIGS

Major Field: ANIMAL SCIENCE

Abstract: Tryptophan (Trp), Isoleucine (Ile) and valine (Val) play important roles on variety of physiological processes, but their mechanisms of action on regulation of growth and nutrients metabolism are not fully understood. The objective of study 1 was to assess the effect of dietary Trp on glucose and fat metabolism in a low birthweight piglet model. Seven-day-old piglets were assigned to 4 treatments: normal birthweight-0%Trp, Low Birth Weight-0%Trp (LBW-T0), LBW-0.4%Trp, and LBW-0.8%Trp for 3 weeks. The results of study 1 showed that Trp improved the hepatic lipid and glucose metabolism by reducing the lipogenesis, gluconeogenesis, and glucose efflux and increasing the lipolysis and glycolysis in LBW piglets. The objective of study 2 was to investigate the effect of a mixture of Val above and Ile at NRC levels on growth, nutrient digestibility and gut microbiota in pigs fed with very low protein (VLP) diets. Forty, three-week-old weaned piglets were randomly assigned to following dietary groups (8 pigs/group) for 5 weeks: positive control (PC): normal protein diet; negative control (NC): VLP diet with first four limiting amino acids (FFL, i.e., Lys, Met, Thr, and Trp) at NRC levels; VA: NC with Val above NRC; IL: NC with Ile at NRC level; VAIL: NC with Val above and Ile at NRC levels. In this study we showed that VAIL partially improved the growth performance which is likely linked with alterations in gut microbiota composition. The objective of study 3 was to investigate the effect of dietary Val and Ile on gene and protein expression of key rate limiting enzymes of urea cycle and lipid metabolism in pigs fed with VLP diets. Forty, three-week-old, weaned barrows were assigned to 5 treatments: 1) PC: normal protein diet; 2) NC: VLP diet containing FFL at NRC levels; 3) HV: NC with Val above NRC; 4) HI: NC with Ile above NRC; 5) HVI: NC with both Val and Ile above NRC. The results of study 3 demonstrated that a combination of dietary Val and Ile improved the growth, feed intake, and glucose clearance and decreased the rate of lipogenesis induced by VLP diets.

TABLE OF CONTENTS

Chapter Page

Chapter Page

Chapter Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

1.1. Large neutral amino acids (LNAA)

Amino acids (AA) were fundamental elements for the formation of early life. The existence of AA in extraterrestrial sources like meteorites [1] supported the fact that a significant evolutionary step resulted in ability to combine AA to form peptides and eventually proteins, which regulate biological processes [2]. Each AA has a carbon atom connected to a carboxyl group and an amino group as well as a distinct side chain, which confers various biological qualities and abilities [3]. Initially, 10 AA were suggested as proteinogenic AA including alanine, aspartate, glutamate, glycine, branched-chain AA (BCAA; isoleucine, leucine, valine), proline, serine, and threonine (Thr) [4]. Later, the sulfurous (methionine (Met)) and aromatic AA (tryptophan (Trp) and phenylalanine (Phe)) emerged [5]. Despite hundreds of AA in nature, mammals can only use 20 AA for protein synthesis [6]. The 20 proteogenic AA are categorized into essential AA, which cannot be sufficiently synthesized in the body and must be obtained through the diet, and non-essential AA which can be sufficiently synthesized in the body, depending on physiological conditions [2, 6, 7].

Among essential AA, Trp, BCAA, Phe, Thr, Met, histidine (His) and from conditionally essential/non-essential AA tyrosine (Tyr) are classified as a large neutral AA (LNAA) [8]. LNAA compete for their transporters and imbalance between these AA can lead to a reduction in feed intake [9, 10]. LNAA are not only crucial for protein synthesis and growth in both humans and animals, but also, they are involved in the metabolism of proteins, lipids, and carbohydrates.

1.2. Role of LNAA on metabolic health and growth

Tryptophan is one of the LNAA that is crucial for growth in animals [11-15] and humans [15-17]. Tryptophan plays variety of roles in physiologic and pathophysiologic processes and has complex impact on health [18]. Tryptophan is necessary for the production of biogenic amines, proteins, and the metabolism of energy. The majority of Trp metabolites have several local and systemic functions and are physiologically and chemically active. Therefore, Trp dysregulation has been linked to a number of pathologic conditions, such as diabetes, inflammatory bowel diseases, cancer, cardiovascular diseases, and central nervous system diseases [18].

Similar to Trp, BCAA are important for the growth [19]. Branched chain AA signals have the power to directly or indirectly control a range of metabolic processes, including appetite, energy production, AA uptake, and immunity [20]. The balance of AA, particularly the balance of BCAA, is crucial for the healthy growth of humans and other animals because these AA play a key function in regulating signal pathways and cell metabolism [21].

Amino acids Phe and Tyr are required for the growth as well [22, 23]. The liver and kidneys can irreversibly hydroxylate Phe to produce Tyr, and the Phe supply activates the phenylalanine hydroxylase enzyme that catalyzes the conversion [24]. Tyrosine serves as a precursor for thyroid hormones, catecholamines, and melanin as well as being involved in protein synthesis [25]. Tyrosine is regarded as a conditionally essential amino acid since a Tyr shortfall can happen if the dietary Tyr supply is low or if there is not enough dietary Phe to synthesize Tyr [25]. The minimal and maximal

Phe requirements for humans have been established, e.g., when the diet is Tyr-free, the Phe requirement is the highest, and some of the Phe must be converted to Tyr to meet the Tyr requirement. In contrast, when the Tyr requirement is met, the Phe requirement is minor [23, 26]. Phenylalanine has been also reported as a necessary amino acid for the formation of neurotransmitters (norepinephrine, epinephrine, and dopamine), and regulates cholecystokinin secretion [27].

Histidine is an indispensable amino acid in poultry, mammals, fish, and in some studies reported in ruminants [3, 28, 29]. Histidine has various roles in the histaminergic system, erythropoiesis, reactive oxygen, and nitrogen species scavenging, metal ion chelation, and proton buffering [30]. The antioxidant, anti-inflammatory, and anorexigenic characteristics of His might be beneficial for treating the metabolic syndrome. Histidine has been reported to decrease oxidative stress and generation of inflammatory cytokines, reactive oxygen species per reactive nitrogen species, and advanced glycation end products per advanced lipoxidation end products [30]. Histidine has beneficial effects on neurological disorders, atopic dermatitis, metabolic syndrome, diabetes, uraemic anaemia, ulcers, inflammatory bowel diseases, malignancies, and muscle performance during strenuous exercise [30].

Threonine or α-amino-β-hydroxybutyric acid was shown to be the third limiting amino acid in corn-soybean meal-based diets for broilers and the second limiting amino acid for finishing pigs fed a corn-soybean meal-based diet [31, 32]. The Thr has a key mediating role in the processes of protein synthesis, energy metabolism, and nutrition absorption [32-34]. Appropriate levels of dietary Thr have been shown to support animal growth, improve immunological function, and preserve intestinal health [35, 36].

Methionine is a sulfur containing AA which is the second or third limiting amino acid in corn-soybean meal diets for pigs and the first limiting amino acid in diets for poultry in maize-soybean based diets [37-39]. Methionine is involved in protein synthesis, methyl donation [40], antioxidant properties [41,

42], and serves as a precursor to bioactive substances including glutathione and taurine [40] which are critical to animal development and health status [43, 44].

Animals' growth, development, and health are influenced by the interactions of all nutrients with one another [45]. LNAA may enhance the health, survival, growth, and development of animals through alterations in carbohydrate, lipid, and protein metabolism.

1.3. LNAA: effects on glucose, lipid, and protein metabolism

Tryptophan has been demonstrated to control the insulin and incretin secretion in animal studies [46]. The intragastric infusion of Trp with a glucose load lowers the blood glucose levels, most likely due to increased concentration of insulinotropic polypeptide [47]. Moreover, it has been demonstrated that Trp metabolite, 3-hydroxyanthranilic, reduce plasma lipid levels including triglycerides, total cholesterol, and very-low-density lipoprotein (VLDL) [48]. Tryptophan has been shown to reduce the protein degradation and improve the N utilization efficiency in pigs [49].

Branched chain amino acids have a significant role in the regulation of lipid metabolism, insulin sensitivity and the mTOR (mammalian target of rapamycin) pathway [21]. Prior studies have shown that BCAA, particularly Leu, can suppress the intracellular lipid accumulation in adipocytes via stimulating mitochondrial biogenesis [50]. Multiple studies have shown that BCAA stimulate insulin secretion and upregulate the glucose transporters. Paradoxically, it has been shown high dietary BCAA levels increase the hyperphagia and obesity due to relative changes in the ratio of other AA specifically Trp and Thr [20]. Furthermore, recent studies indicate that each BCAA has varied impacts on glucose utilization and insulin sensitivity [51]. Further investigation is required to fully understand the effects of BCAA on glucose and lipid metabolism.

Methionine, Phe, and Val stimulate the leptin secretion from adipocytes [48] and influence the fat metabolism [52]. Phenylalanine ingestion led to higher levels of glucagon and insulin and had an impact on glucose metabolism [53]. In AML12 hepatocytes, Ile, Phe, and His may change the extracellular

acidification rates, which is an indirect indicator of glycolysis [54]. Tyrosine metabolism has an impact on endogenous lipid synthesis pathway since its end products such as fumarate and acetoacetate are significant sources to convert to acetyl-CoA [55]. Through methylation, His controls both biological function and gene expression of proteins [3]. It has been demonstrated that rats fed with an excess dietary His lowered the blood cholesterol more than those fed with basal diet [56]. Dietary His reduced hepatic triglyceride and cholesterol as well as lipogenesis and cholesterol metabolism [57]. Additionally, His supplementation has been shown to increase the insulin sensitivity and reduce the hyperinsulinemia [58, 59].

Threonine has been shown to enhance the serum lipid profile which shows the role of Thr on regulation of lipid metabolism. Threonine lowered the serum glucose, triacylglycerols, total cholesterol, and low-density lipoprotein (LDL)-cholesterol concentrations relative to high fat diets [60]. Methionine's effect on lipid metabolism has also been addressed, primarily as a strategy for lowering insulin resistance, obesity, and type 2 diabetes [61]. Among LNAA, inconsistent results have been reported in the literature for the effect of Trp, Val and Ile on lipid, glucose and protein metabolism and that requires further research to be fully understood.

1.4. References

1. Burton, A.S.; Stern, J.C.; Elsila, J.E.; Glavin, D.P.; Dworkin, J.P. Understanding prebiotic chemistry through the analysis of extraterrestrial amino acids and nucleobases in meteorites. Chem. Soc. Rev. 2012, 41, 5459– 5472. DOI: 10.1039/C2CS35109A

2. Kelly, B.; Pearce, E.L. Amino assets: how amino acids support immunity. Cell Metab. 2020, 32, 154– 175. DOI: 10.1016/j.cmet.2020.06.010

3. Wu, G. Amino acids: metabolism, functions, and nutrition. Amino acids. 2009, 37, 1– 17. DOI: 10.1007/s00726-009-0269-0

4. Doi, N.; Kakukawa, K.; Oishi, Y.; Yanagawa, H. High solubility of random-sequence proteins consisting of five kinds of primitive amino acids. Protein Eng. Des. Sel. 2005, 18, 279–284. DOI: 10.1093/protein/gzi034

5. Longo, L.M.; Tenorio, C.A.; Kumru, O.S.; Middaugh, C.R.; Blaber, M. A single aromatic core mutation converts a designed “primitive” protein from halophile to mesophile folding. Protein Sci. 2015, 24, 27–37. DOI: 10.1002/pro.2580

6. Lopez, M.J.; Mohiuddin, S.S. Biochemistry, essential amino acids; In: StatPearls Publishing: Treasure Island, USA, 2022.

7. Wu, G. Functional amino acids in nutrition and health. Amino acids. 2013, 45, 407–411. DOI: 10.1007/s00726-013-1500-6

8. Van Spronsen, F.J.; Hoeksma, M.; Reijngoud, D.J. Brain dysfunction in phenylketonuria: is phenylalanine toxicity the only possible cause? J. Inherit. Metab. Dis. 2009, 32, 46–51. DOI: 10.1007/s10545-008-0946-2

9. Andersen, A.E.; Avins, L. Lowering brain phenylalanine levels by giving other large neutral amino acids: a new experimental therapeutic approach to phenylketonuria. Arch. Neurol. 1976, 33, 684– 686. DOI: 10.1001/archneur.1976.00500100018008

10. Tackman, J.M., Tews, J.K. and Harper, A.E. Dietary disproportions of amino acids in the rat: effects on food intake, plasma and brain amino acids and brain serotonin. J. Nutr. 1990, 120, 521–533. DOI: 10.1093/jn/120.5.521

11. de Marte, M.L.; Enesco, H.E. Influence of low tryptophan diet on survival and organ growth in mice. Mech. Ageing Dev. 1986, 36, 161–171. DOI: 10.1016/0047-6374(86)90017-5

12. Eder, K.; Peganova, S.; Kluge, H. Studies on the tryptophan requirement of piglets. Arch. Anim. Nutr. 2001, 55, 281–279. DOI: 10.1080/17450390109386198

13. Shen, Y.B.; Voilqué, G.; Kim, J.D.; Odle, J.; Kim, S.W. Effects of increasing tryptophan intake on growth and physiological changes in nursery pigs. J. Anim. Sci. 2012, 90, 2264– 2275. DOI: 10.2527/jas.2011-4203

14. Segall, P.E.; Timiras, P.S. Patho-physiologic findings after chronic tryptophan deficiency in rats: a model for delayed growth and aging. Mech. Ageing Dev. 1976, 5, 109–124. DOI: 10.1016/0047-6374(76)90012-9

15. Moehn, S.; Pencharz, P.B.; Ball, R.O. Lessons learned regarding symptoms of tryptophan deficiency and excess from animal requirement studies. J. Nutr. 2012, 142, 2231S–2235S. DOI: 10.3945/jn.112.159061

16. Joint, F.A.O.; World Health Organization. Protein and amino acid requirements in human nutrition: report of a joint FAO/WHO/UNU expert consultation. W. H. O. 2007.

17. WHO, J. Protein and amino acid requirements in human nutrition. W. H. O. Tech. Rep. Series. 2007.

18. Comai, S.; Bertazzo, A.; Brughera, M.; Crotti, S. Tryptophan in health and disease. Adv. Clin. Chem. 2020, 95, 165–218. DOI: 10.1016/bs.acc.2019.08.005

19. Ren, M.; Zhang, S.H.; Zeng, X.F.; Liu, H.; Qiao, S.Y. Branched-chain amino acids are beneficial to maintain growth performance and intestinal immune-related function in weaned piglets fed protein restricted diet. Asian-australas. J. Anim. Sci. 2015, 28, 1742. DOI: 10.5713/ajas.14.0131

20. Solon-Biet, S.M.; Cogger, V.C., Pulpitel, T.; Wahl, D.; Clark, X.; Bagley, E.E.; Gregoriou, G.C.; Senior, A.M.; Wang, Q.P.; Brandon, A.E. Perks, R. Branched-chain amino acids impact health

and lifespan indirectly via amino acid balance and appetite control. Nat. Metab. 2019, 1, 532–545. DOI: 10.1038/s42255-019-0059-2

21. Zhang, L.; Li, F.; Guo, Q.; Duan, Y.; Wang, W.; Yang, Y.; Yin, Y.; Gong, S.; Han, M.; Yin, Y. Different proportions of branched-chain amino acids modulate lipid metabolism in a finishing pig model. J. Agric. Food Chem. 2021, 69, 7037–7048. DOI: 10.1021/acs.jafc.1c02001

22. Mertz, E.T.; Beeson, W.M.; Jackson, H.D. Classification of essential amino acids for the weanling pig. Arch. Biochem. Biophys. 1952, 38, 121–128. DOI: 10.1016/0003-9861(52)90015-5

23. Gloaguen, M.; Le Floc'h, N.; Primot, Y.; Corrent, E.; van Milgen, J. Performance of piglets in response to the standardized ileal digestible phenylalanine and tyrosine supply in low-protein diets. Animal. 2014, 8, 1412– 1419. DOI: 10.1017/S1751731114001360

24. Fitzpatrick, P.F. Allosteric regulation of phenylalanine hydroxylase. Arch. Biochem. 2012, 519, 194– 201. DOI: 10.1016/j.abb.2011.09.012

25. Hsu, J.W.; Ball, R.O.; Evidence that phenylalanine may not provide the full needs for aromatic amino acids in children. Pediatr. Res. 2007, 61, 361– 365. DOI: 10.1203/pdr.0b013e318030d0db

26. Pencharz, P.B.; Hsu, J.W.; Ball, R.O. Aromatic amino acid requirements in healthy human subjects. J. Nutr. 2007, 137, 1576S– 1578S. DOI: 10.1093/jn/137.6.1576S

27. Akram, M.; Daniyal, M.; Ali, A.; Zainab, R.; Shah, S.M.A.; Munir, N.; Tahir, I.M. In Synucleins-Biochemistry and Role in Diseases; Surguchov, A., Eds.; IntechOpen, London., England, 2020; pp. 96. DOI: 10.5772/intechopen.73750

28. Rose, W.C. Nutrition Classics. Federation Proceedings 8 (2) 546-52, June 1949. Amino acid requirements of man. William C. Rose. Nutr. Rev. 1976, 34, 307–309. DOI: 10.1111/j.1753-4887.1976.tb05679.x

29. Onodera, R. Essentiality of histidine in ruminant and other animals including human beings. Asian-australas. J. Anim. Sci. 2003, 34, 445–454. DOI: 10.5713/ajas.2003.445

30. Holeček, M. Histidine in health and disease: metabolism, physiological importance, and use as a supplement. Nutrients 2020, 12, 848. DOI: 10.3390/nu12030848

31. Karau, A.; Grayson, I. Amino Acids in Human and Animal Nutrition. In Biotechnology of Food and Feed Additives. Advances in Biochemical Engineering/Biotechnology; Zorn, H., Czermak, P. Eds.; Springer: Berlin, Heidelberg, Germany, 2014; Volume 143, pp. 189–228. DOI: 10.1007/10_2014_269

32. Chen, Y.P., Cheng, Y.F., Li, X.H., Yang, W.L., Wen, C., Zhuang, S. and Zhou, Y.M. Effects of threonine supplementation on the growth performance, immunity, oxidative status, intestinal integrity, and barrier function of broilers at the early age. Poult. Sci.2017, 96, 405–413. DOI: 10.3382/ps/pew240

33. Estalkhzir, F.M.; Khojasteh, S.; Jafari, M. The effect of different levels of threonine on performance and carcass characteristics of broiler chickens. J. Nov. Appl. Sci. 2013, 2, 382–386.

34. Tang, Q.; Tan, P.; Ma, N.; Ma, X. Physiological functions of threonine in animals: beyond nutrition metabolism. Nutrients. 2013, 13, 2592. DOI: 10.3390/nu13082592

35. Wang, W.; Zeng, X.;Mao, X.; Wu, G.; Qiao, S. Optimal dietary true ileal digestible threonine for supporting the mucosal barrier in small intestine of weanling pigs. J. Nutr. 2010, 140, 981–986. DOI: 10.3945/jn.109.118497

36. Chen, Y.; Zhang, H.; Cheng, Y.; Li, Y.; Wen, C.; Zhou, Y. Dietary l-threonine supplementation attenuates lipopolysaccharide-induced inflammatory responses and intestinal barrier damage of broiler chickens at an early age. Br. J. Nutr. 2018, 119, 1254–1262. DOI: 10.1017/S0007114518000740

37. Cromwell, G.L. Identifying the limiting amino acids in complex and cereal grain-based diets to minimize nitrogen excretion. In Midwest Swine Nutrition Conference Nutrition Conference Proceedings. Indianapolis, IN, USA, The Ohio Univ. Press, Columbus, OH, USA; 2004, 69–83.

38. Dilger, R.N.; Baker, D.H. DL-Methionine is as efficacious as L-methionine, but modest L-cystine excesses are anorexigenic in sulfur amino acid-deficient purified and practical-type diets fed to chicks. Poult. Sci. 2007, 86, 2367–2374. DOI: 10.3382/ps.2007-00203

39. Shen, Y.B.; Weaver, A.C.; Kim, S.W. Effect of feed grade L-methionine on growth performance and gut health in nursery pigs compared with conventional DL-methionine. J. Anim. Sci. 2014, 92, 5530–5539. DOI: 10.2527/jas.2014-7830

40. Finkelstein, J.D. Methionine metabolism in mammals. J. Nutr. Biochem. 1990, 1, 228– 237. DOI: 10.1016/0955-2863(90)90070-2

41. Reddy, V.Y.; Desorchers, P.E.; Pizzo, S.V.; Gonias, S.L.; Sahakian, J.A.; Levine, R.L.; Weiss, S.J. Oxidative dissociation of human alpha 2-macroglobulin tetramers into dysfunctional dimers. J. Biol. Chem. 1994, 269, 4683–4691. DOI: 10.1016/S0021-9258(17)41830-8

42. Luo, S. and Levine, R.L. Methionine in proteins defends against oxidative stress. FASEB. J. 2009, 23, 464–472. DOI: 10.1096/fj.08-118414

43. Stoll, B.; Henry, J.; Reeds, P.J.; Yu, H.; Jahoor, F.; Burrin, D.G. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. J. Nutr. 1998, 128, 606–614. DOI: 10.1093/jn/128.3.606

44. Riedijk, M.A.; Stoll, B.; Chacko, S.; Schierbeek, H.; Sunehag, A.L., van Goudoever, J.B.; Burrin, D.G. Methionine transmethylation and transsulfuration in the piglet gastrointestinal tract. Proc. Natl. Acad. Sci. 2007, 104, 3408–3413. DOI: 10.1073/pnas.0607965104

45. Wu, G. Nutrition and Metabolism: Foundations for Animal Growth, Development, Reproduction, and Health. In Recent Advances in Animal Nutrition and Metabolism; Wu, G. Eds.; Springer: Berlin, Heidelberg, Germany, 2022; Volume 1354, pp. 1–24. DOI: 10.1007/978-3-030-85686-1_1

46. Kiluk, M., Lewkowicz, J., Pawlak, D. Tankiewicz-Kwedlo, A. Crosstalk between Tryptophan Metabolism via Kynurenine Pathway and Carbohydrate Metabolism in the Context of Cardio-Metabolic Risk. J. Clin. Med. 2021, 10, 2484. DOI: 10.3390/jcm10112484

47. Ponter, A.A., Sève, B. and Morgan, L.M. Intragastric tryptophan reduces glycemia after glucose, possibly via glucose-mediated insulinotropic polypeptide, in early-weaned piglets. J. Nutr. 1994, 124, 259–267. DOI: 10.1093/jn/124.2.259

48. Zhang, L.; Ovchinnikova, O.; Jönsson, A.; Lundberg, A.M.; Berg, M.; Hansson, G.K.; Ketelhuth, D.F. The tryptophan metabolite 3-hydroxyanthranilic acid lowers plasma lipids and decreases atherosclerosis in hypercholesterolaemic mice. Eur. Heart J. 2012, 33, 2025–2034. DOI: 10.1093/eurheartj/ehs175

49. Nørgaard, J.V.; Pedersen, T.F.; Soumeh, E.A.; Blaabjerg, K.; Canibe, N.; Jensen, B.B.; Poulsen, H.D. Optimum standardized ileal digestible tryptophan to lysine ratio for pigs weighing 7–14 kg. Livest. Sci. 2015, 175, 90–95. DOI: 10.1016/j.livsci.2015.02.012

50. Sun, X.; Zemel, M.B. Leucine modulation of mitochondrial mass and oxygen consumption in skeletal muscle cells and adipocytes. Nutr. Metab. 2009, 6, 1–8. DOI: 10.1186/1743-7075-6-26

51. White, P.J.; Lapworth, A.L.; An, J.; Wang, L.; McGarrah, R.W.; Stevens, R.D.; Ilkayeva, O.; George, T.; Muehlbauer, M.J.; Bain, J.R.; Trimmer, J.K. Branched-chain amino acid restriction in Zucker-fatty rats improves muscle insulin sensitivity by enhancing efficiency of fatty acid oxidation and acyl-glycine export. Mol. Metab. 2016, 5, 538–551. DOI: 10.1016/j.molmet.2016.04.006

52. Cammisotto, P.G., Gélinas, Y., Deshaies, Y. and Bukowiecki, L.J. Regulation of leptin secretion from white adipocytes by insulin, glycolytic substrates, and amino acids. Am. J. Physiol. Endocrinol. 2005, 289, E166– E171. DOI: 10.1152/ajpendo.00602.2004

53. Nuttall, F.Q.; Schweim, K.J.; Gannon, M.C. Effect of orally administered phenylalanine with and without glucose on insulin, glucagon and glucose concentrations. Horm. Metab. Res. 2006, 38, 518–523. DOI: 10.1055/s-2006-949523

54. Suzuki, R.; Sato, Y.; Fukaya, M.; Suzuki, D.; Yoshizawa, F.; Sato, Y. Energy metabolism profile of the effects of amino acid treatment on hepatocytes: phenylalanine and phenylpyruvate inhibit glycolysis of hepatocytes. Nutrition 2021, 82, 111042. DOI: 10.1016/j.nut.2020.111042

55. Wang, J.; Qiao, Y.; Sun, H.; Chang, H.; Zhao, H.; Zhang, S.; Shan, C. Decreased SLC27A5 Suppresses Lipid Synthesis and Tyrosine Metabolism to Activate the Cell Cycle in Hepatocellular Carcinoma. Horm. Metab. Res. 2022, 10, 234. DOI: 10.3390/biomedicines10020234

56. Xu, H.; Sakakibara, S.; Morifuji, M.; Salamatulla, Q.; Aoyama, Y. Excess dietary histidine decreases the liver copper level and serum alanine aminotransferase activity in Long-Evans Cinnamon rats. Br. J. Nutr. 2003, 90, 573–579. DOI: 10.1079/BJN2003939

57. Mong, M.C.; Chao, C.Y.; Yin, M.C. Histidine and carnosine alleviated hepatic steatosis in mice consumed high saturated fat diet. Eur. J. Pharmacol. 2011, 653, 82–88. DOI: 10.1016/j.ejphar.2010.12.001

58. DiNicolantonio, J.J.; McCarty, M.F.; OKeefe, J.H. Role of dietary histidine in the prevention of obesity and metabolic syndrome. Open Heart. 2018, 5, e000676. DOI: 10.1136/openhrt-2017-000676

59. Feng, R.N.; Niu, Y.C.; Sun, X.W.; Li, Q.; Zhao, C.; Wang, C.; Guo, F.C.; Sun, C.H.; Li, Y. Histidine supplementation improves insulin resistance through suppressed inflammation in obese

women with the metabolic syndrome: a randomised controlled trial. Diabetologia 2013, 56, 985–994. DOI: 10.1007/s00125-013-2839-7

60. Ma, Q.; Zhou, X.; Sun, Y.; Hu, L.; Zhu, J.; Shao, C.; Meng, Q.; Shan, A. Threonine, but not lysine and methionine, reduces fat accumulation by regulating lipid metabolism in obese mice. J. Agric. Food Chem. 2020, 68, 4876–4883. DOI: 10.1021/acs.jafc.0c01023

61. Martínez, Y.; Li, X.; Liu, G.; Bin, P.; Yan, W.; Más, D.; Valdivié, M.; Hu, C.A.A.; Ren, W.; Yin, Y. The role of methionine on metabolism, oxidative stress, and diseases. Amino acids. 2017, 49, 2091–2098. DOI: 10.1007/s00726-017-2494-2

CHAPTER II

REVIEW OF LITERATURE

Among LNAA, Trp, Ile and Val play important roles on variety of physiological processes including lipid, carbohydrate, and protein metabolism. Therefore, the objective of this literature review is to summarize the 1) digestion, absorption, and metabolism of fats, carbohydrates, and proteins, 2) role of Trp, Val and Ile on protein, carbohydrate, and lipid metabolism.

2.1. Digestion, absorption, and metabolism of fats

2.1.1. Fat digestion

Triglycerides, phospholipids, cholesterol, and cholesterol esters are the main dietary lipids; nevertheless, triglycerides are nearly 90% of total dietary lipids [1]. Triglycerides need to be broken down into free fatty acids and monoglycerides in order to be absorbed [2, 3]. Lingual lipase of saliva and gastric lipase synthesized by the chief cells of stomach are involved in initial steps of fats digestion [2]. Fats emulsification in the small intestine facilitates the function of pancreatic lipase on fat droplets [1]. During emulsification large lipid globules are split into smaller droplets with a high surface area to volume ratio [1]. The primary emulsifiers are bile acids and the phospholipid lecithin, both of which are produced in the liver and secreted through bile duct into small intestine [1, 2].

Although gastric lipase is released into the stomach, its role in fat digestion is secondary to the enzymatic processes that take place in the small intestine in association with bile salts [2, 3]. Enteroendocrine cells in the duodenal epithelium detect the release of chyme into the duodenum and produce hormones that direct processes leading to lipid breakdown [3]. The release of secretin from enteroendocrine S-cells is initiated by the chyme's acidity. When secretin acts on the pancreatic ducts, bicarbonate-rich secretions are released, which increase the pH of the duodenal contents for optimal function of digestive enzymes. Cholecystokinin (CCK) is released into the circulation by enteroendocrine I-cells in response to lipids in the lumen, and this is the main signal for pancreatic acinar cells to secrete their digestive enzymes. Moreover, CCK causes gallbladder contraction, which releases bile salts into the lumen, delays gastric emptying for the optimum lipid digestion, and activates the CCK1 receptor on afferent vagal neurons. Although gastric and lingual lipases continue to function in the small intestine and work in concert with pancreatic lipase, up to 70% of the hydrolysis of triglycerides is carried out by pancreatic lipase, the main enzyme involved in triglyceride digestion [3]. The protein colipase stabilizes lipase against surface denaturation and improves its attaching to lipid emulsions; therefore, it is necessary for pancreatic lipase activity [2-5]. Further, pancreatic cholesterol esterase breaks down cholesterol esters and produce fatty acid and cholesterol and the pancreatic phospholipase A2 disassembles phospholipid into its fatty acid and phosphate group attached to glycerol backbone [1].

2.1.2. Fat absorption and metabolism

In the state of health, the digestive tract effectively absorbs 95% of the consumed fat; the remaining 5% is expelled in feces [3]. After being broken down by pancreatic lipase, a part of the triglycerides and fatty acids may condense into vesicles before transport to the intestinal mucosa [6]. As free fatty acids are formed, they combine with the bile salts to form micelles with a diameter of around 2 nm [3]. Micelles facilitate fatty acids absorption in the small intestine by bringing hydrophobic lipid digestion products close enough to the brush border [1]. Through the enterocyte membrane,

micelles and fatty acids are absorbed by passive diffusion, facilitated diffusion, and active transport [7]. Bile salts mainly reabsorbed in the terminal ileum and then transferred to the liver (enterohepatic recirculation) [3]. Fatty Acid Transporter 4 and cluster of differentiation 36 (CD36), two proteins that are known to transport fatty acids, are expressed apically in jejunal enterocytes, which may enhance the transfer of fatty acids from the lumen into the epithelium [3].

In the bloodstream, large concentrations of free fatty acids and cholesterol are toxic, therefore, they must be converted to triglycerides and cholesterol esters in the cytoplasm of enterocytes [1]. Fatty acids bind to a protein called fatty-acid binding protein in the cytosol, which moves them from the cell membrane to the smooth endoplasmic reticulum (ER) to form triglycerides and phospholipids from fatty acids and monoglycerides [7]. Triglycerides and apolipoproteins then combined in rough ER to form chylomicrons, which are transported to the Golgi apparatus to exit with secretory vesicle out of the cell [2, 3, 7]. Chylomicrons are solubilized by apolipoprotein component, but due to their size, they cannot penetrate capillaries and must instead enter the systemic circulation through the lymphatics. Shorter chain lipids are soluble in the blood in the absence of apolipoprotein, and they simply transferred into capillaries [3].

Triacylglycerol is delivered by chylomicrons mostly to peripheral tissues where it is stored or used as fuel and the remaining lipid is delivered to liver [2]. Triacylglycerol must go through lipolysis by lipoprotein lipase (LPL) within blood vessels before its uptake [8]. Lipoprotein lipase controls plasma triacylglycerol concentration through hydrolyzing VLDL and chylomicrons [9]. LPL is expressed in the heart, skeletal muscle, brown adipose tissue, and white adipose tissue that oxidize or store substantial amounts of fatty acids. The protein GPI-anchored protein HDL binding protein transfers LPL and then binds it to the capillary endothelium [10, 11]. The hydrolytic cleavage of triglycerides into fatty acids and glycerol is catalyzed by LPL [12]. Free fatty acid and diacylglycerol absorbed by tissues [2]. The part of chylomicron that is left is called chylomicron remnant which is removed from circulation by hepatocytes [2].

The absorbed glycerol in tissues is used through glycolysis for energy and gluconeogenesis for glucose production. Fatty acids are first activated by acyl-CoA synthase by formation of fatty acyl-CoA. Short chain fatty acids pass through mitochondria without carriers while long chain fatty acids pass to mitochondrial matrix via carnitine palmitoyltransferase 1 (CPT1), carnitine palmitoyltransferase 2 (CPT2) and translocase. Then, fatty acyl-CoA goes through beta oxidation and produce acetyl-CoA [2]. Hormone sensitive lipase (HSL) is a key enzyme for lipolysis that is stimulated by epinephrine, norepinephrine, adrenocorticotropic hormone (ACTH), thyroid stimulating hormone, glucagon and growth hormone while insulin inhibits its activity [2]. Lipogenesis is a metabolic process by which acetyl-CoA carboxylase (ACC) first converts acetyl CoA into malonyl-CoA, and then fatty acid synthase (FAS) uses acetyl-CoA, malonyl-CoA, and NADPH to create fatty acids [13]. In the last step of lipogenesis, three free fatty acids and one glycerol are combined to form a triglyceride with diacylglycerol acyltransferase [13]. There are several regulators controlling the lipogenesis process. For example, the membrane-bound transcription factor sterol regulatory element-binding proteins (SREBPs) significantly upregulate the genes involved in the generation and absorption of fatty acids [14]. Additionally, nuclear receptor peroxisome proliferator-activated receptors (PPARs) enhance fatty acid synthesis by stimulating the expression of acyl-CoA synthetase and fatty acid transport protein [15].

2.2. Digestion, absorption, and metabolism of carbohydrate

2.2.1. Carbohydrate digestion

Carbohydrate digestion starts from oral cavity with the function of salivary amylase, which is secreted by the parotid and submandibular glands, and breaks starch into simpler disaccharides and trisaccharides. Salivary amylase is inactive at the stomach's acidic pH. Pancreatic α-amylase hydrolyzes the residual polysaccharides upon passage into the duodenum to produce disaccharides and trisaccharides [1-3]. Maltose, maltotriose (isomaltose), trisaccharides, oligosaccharides, and α-limit dextrins are formed as a result of the breakdown of complex carbohydrates by pancreatic α-

amylase [16]. Prior to the absorption, di-, tri-, and oligosaccharides are digested by brush border enzymes [17]. Maltase, a member of the brush-border membrane enzyme family, hydrolyzes the α-1,4-glycosidic links between the glucose molecules in maltose [16]. Limit dextrinase, also referred as isomaltase, hydrolyzes α-1,6-glycosidic bonds at the branch points of certain limit dextrins and α-1,4-glycosidic links in maltose and maltotriose [17]. Sucrase breaks sucrose by hydrolyzing the α-1,2-glycosidic bonds between glucose and fructose. Glucosyl-ceramidase breaks β-glycosidic bonds between hydrophobic residues like those in the glycolipid's glucosylceramide and galatosylceramide and glucose or galactose. Lactase breaks the β-1,4 bonds between glucose and galactose [16].

2.2.2. Carbohydrate absorption and metabolism

Epithelial cells can only absorb monosaccharides like glucose, galactose, and fructose. These molecules are absorbed in intestine through two primary transporter proteins, sodium-glucose transport protein-1 (SGLT1) and glucose transport protein-5 (GLUT5). Galactose and glucose are both absorbed by SGLT-1, which is powered by the energy of a strong extracellular Na+ gradient. Two Na+ are transferred for every glucose or galactose molecule. The Na+/K+-ATPase pumps in the membrane of enterocytes, which pump Na+ back out of the cell, keep the gradient. Further, fructose is transported by GLUT5 without the need for sodium gradient [2, 3]. The glucose transport protein-2 (GLUT-2), a cotransporter for all three monosaccharides, then allows monosaccharides to exit the cell basolaterally and reach the bloodstream [1-3]. The portal vein and hepatic artery carry glucose, galactose and fructose to the hepatic cells from the intestine and the systemic circulation, respectively [18].

In many cell types, such as the brain and epithelial layers, GLUT-1 is in charge of supplying a basic level of glucose. GLUT-2, which is mostly present in liver and kidney cells, in addition to absorbing glucose, may also transport glucose into the blood when plasma concentrations are low [19]. The

perfect synchronization of glucose utilization and endogenous glucose synthesis or dietary glucose intake is necessary to maintain a normal plasma glucose level [20]. Three processes regulate blood glucose level including glycogenolysis, gluconeogenesis, and intestinal absorption of dietary carbohydrates. Glucose takes several pathways in cells including storage as glycogen, conversion to pyruvate during glycolysis, and release into the bloodstream by the liver and kidneys. Liver and kidney are the organs that contains the enzyme glucose-6-phosphatase required for the release of glucose into the bloodstream [20]. Plasma glucose concentration is regulated by the rate of glucose entering the circulation (glucose appearance) and the rate of glucose removal from circulation (glucose disappearance) [21]. Glucoregulatory hormones including insulin, glucagon, amylin, glucagon like peptide-1, gastric inhibitory peptide (GIP), epinephrine, cortisol and growth hormone contribute to maintain circulating glucose concentration [22]. Insulin is an anabolic hormone that is secreted from pancreatic β cell in response to high blood glucose and AA in the fed state. Insulin receptors in many cells such as fat, liver, and muscle regulate postprandial glucose levels by stimulating glucose disappearance [22].

Liver is one of the main organs that regulates glucose metabolism through glycogenesis, and glycolysis in fed state, and gluconeogenesis, and glycogenolysis in fast state [2]. Inside liver cells free glucose is phosphorylated to glucose 6-phosphate (G6P) by glucokinase (GCK) [23]. Multiple metabolic actions can use glucose 6-phosphate, including oxidation to start the pentose phosphate pathway and synthesis of uridine diphosphate (UDP)-glucose and fructose 6-phosphate. UDP-glucose is used to generate the glycogen, UDP-glucuronate, and UDP-galactose [18]. After meals, the majority of the glucose that enters the hepatocyte is converted to glycogen via glycogenesis and stored as a supply of energy that can be used during fasting [24]. There are two rate limiting enzymes for glycogenesis including GCK, and glycogen synthesis which is stimulated by insulin [2]. A series of metabolic pathways, including glycolysis in the cytosol, the tricarboxylic acid (TCA) cycle, and the respiratory chain in the mitochondrial network, convert glucose to carbon

dioxide. In the cytosol, glycolysis converts glucose into pyruvate while also generating a small quantity of ATP and NADH. Glycolysis is regulated by 3 rate limiting enzymes including GCK or hexokinase, phosphofructokinase-1 (PFK-1) and pyruvate kinase (PK). GCK is upregulated by insulin and negatively regulated by glucose-6-phosphate (G6P). Phosphofructokinase-1 is allosterically inhibited by ATP, citrate and is activated by AMP, fructose1,2 bisphosphates. Pyruvate kinase is allosterically activated by AMP and fructose1,6 bisphosphates and is inhibited by ATP, alanine and acetyl-CoA and covalently regulated by glucagon [2, 18]. Glycogenolysis, or the breakdown of glycogen, and de novo glucose synthesis or gluconeogenesis are the sources of hepatic glucose. As the glycogen storage is exhausted, glycogenolysis reduces, making gluconeogenesis the body's main source of glucose [25]. In glycogenolysis pathway glycogen phosphorylase is a rate limiting enzyme which allosterically inhibited by ATP, G6P and is activated by AMP and covalently is activated by glucagon and catecholamine. In gluconeogenesis, the initial process from lactate and alanine takes place inside the mitochondria and involves the carboxylation of pyruvate into oxaloacetate by pyruvate carboxylase (PC) which is allosterically inhibited by ADP and is activated by acetyl-CoA. Then, oxaloacetate is converted to phosphoenolpyruvate through the action of the enzyme phosphoenolpyruvate carboxykinase (PEPCK) which is allosterically inhibited by ADP, and PC [2]. Phosphoenolpyruvate is converted into fructose 1,6-bisphosphate, which can then be turned into fructose 6-phosphate by fructose 1,6-bisphosphatase. Fructose 1,6-bisphosphatase is allosterically inhibited by AMP, fructose 2,6 bisphosphate and is activated by citrate. Glucose 6-phosphate can be produced from fructose 6-phosphate and glucose 6 phosphatase can convert glucose 6-phosphate into glucose [2, 26]. Glucose-6-phosphatase is negatively regulated by its substrate, which is glucose-6-phosphate [2].

2.3. Digestion, absorption, and metabolism of proteins

2.3.1. Protein digestion

Salivary secretions do not contain proteolytic enzymes; thus, stomach is the first site for protein digestion. Pro-enzyme pepsinogen, which is released by the gastric chief cells is activated to pepsin in a low pH environment of stomach. Although pepsin breaks down proteins and polypeptides into smaller peptides; the majority of ingested proteins pass to the small intestine in the form of polypeptides [1, 3]. The release of free AA in the stomach triggers the production of gastric acid, CCK, and secretin and stimulates the emptying of the stomach's contents into the duodenum. In the neutral pH of the small intestine, where pancreatic acinar proteases are the primary enzymes for proteolysis, gastric pepsin becomes inactive [1, 7, 27]. The majority of protein digestion is carried out in the small intestine by pancreatic digestive enzymes. The brush-border enzyme enterokinase is responsible for activating pancreatic trypsinogen. When activated, trypsin can convert trypsinogen to trypsin through an autocatalytic process [1, 7, 27]. Trypsin also converts the other proenzymes of pancreas to their active form. Following their activation, the pancreatic enzymes trypsin, chymotrypsin, elastase, and carboxypeptidase break polypeptides into oligopeptides, which are short chains of several AA [1, 7, 27]. In the small intestine epithelium's brush border, the last phase of protein digestion takes place where oligopeptides are completely broken down by membrane-bound peptidases into single AA, dipeptides, or tripeptides [1-3, 7].

2.3.2. Protein absorption and metabolism

Electrochemical gradients including Na^+ and, occasionally, Cl^- facilitate AA absorption to enterocytes. Further, a Na^+/H^+ exchanger expressed on the luminal enterocyte membrane provides an H^+ concentration gradient that drives the transport of dipeptides and tripeptides. The peptide transporter 1, is in charge of the intestinal peptide transport system. The jejunum has been demonstrated to absorb di- or tripeptides more quickly than free AA. Cytosolic peptidases cleave the dipeptides and tripeptides into individual AA once they enter the enterocytes. Single AA are passively transported from the basolateral membrane into the portal system that will take them to liver or peripheral tissues [1-3, 7, 28]. Uptake of the AA into liver and other tissues take place by

the similar transporters found in intestinal cell membranes such as sodium dependent system SLC7A5, SLC16A, SLC36A, and SLC38A [2, 29]. Liver is the first place for uptake and catabolism of AA except for BCAA which are mainly utilized by muscle and heart [2].

In normal condition at a specific developmental stage, the metabolic pool of free AA in organs, cells, and intracellular organelles remains stable which reflects the delicate balance between the exogenous and endogenous availability of AA and their utilization [28]. The concentrations of AAs in the plasma and tissues do not significantly change during the postabsorptive state [28, 30]. It is notable that free AA concentrations in the plasma and tissues differ depending on the species, stage of development, nutritional status, endocrine status, level of physical activity, time of day, and presence of diseases [31-34].

It is crucial to discuss protein metabolism, including the processes for synthesis and break down of proteins as well as the regulatory mechanisms [35]. Protein synthesis is the process of translating mRNA into protein, carried out by the ribosomes. Through the formation of peptide bonds, the ribosomes catalyze the connection of AA in the new synthesized protein. The three main stages of protein synthesis—initiation, elongation, and termination—are regulated by the phosphorylation or dephosphorylation of several protein factors. Eukaryotic initiation factors (eIFs) are proteins that mediate important steps in initiation of the translation, including the recruitment of the mRNA to the small (40S) ribosome subunit (eIF4) and the recruitment of the initiator methionyl-tRNA (Met-tRNA) which identifies the start codon at the onset of the coding region [36, 37]. Preinitiation complex (40S subunit and Met-tRNA), through a procedure known as scanning, checks 5'-untranslated regions of mRNA for a proper start codon. Eukaryotic elongation factors (eEF1) and eEF2, are necessary for elongation in mammalian cells. The function of eEF2 is to facilitate the ribosome moves by one codon, then the peptidyl-tRNA migrates from the A site to the P site of the ribosome after the formation of a new peptide bond [37, 38]. The terminating codon (e.g., UGA, UAG, or UAA) on the mRNA, detects the end of polypeptide chain elongation in the A site after

several cycles of elongation [28]. Several elements engaged in the initiation and elongation phases of translation are under the regulation of mTOR. Rapamycin prevents insulin, growth hormones, and other growth-promoting agonists from rapidly activating protein synthesis, indicating that mTOR signaling is crucial for stimulating the translational machinery. It has been shown that mTOR signaling regulates the cell's ability to translate via the short- and long-term (minutes and hours, respectively) activation of translation, which leads to increase in the number of ribosomes and other translational components [37]. mTOR or FK506 binding protein 12-rapamycin associated protein 1 has been shown as a principal cell signaling cascade that controls protein synthesis, cytoskeleton remodeling, and autophagy-mediated intracellular protein degradation and is an utmost conserved serine/threonine protein kinase [39, 40]. mTOR is formed by two distinct complexes which are structurally and functionally different in cells including mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [41]. mTORC1 promotes protein synthesis and other anabolic activities to support cellular growth and proliferation as well as inhibition of autophagy by phosphorylating eIF4E-binding protein-1 (4E-BP1) and ribosomal protein S6 kinase-1 (S6K1) [27]. mTORC2 also has been shown to control cell proliferation, differentiation, migration, and cytoskeletal rearrangement by phosphorylating protein kinase B/Akt [42]. Insulin and AA, which activate mTOR, can also activate elongation. The majority of the substantial energy required for protein synthesis is expended during elongation; therefore, inhibiting mTOR through AMPK would phosphorylate and activate eEF2 kinase to slow down the elongation and save energy [43, 44]. In eukaryotic cells, there are two primary pathways for the degradation of proteins: one for cytosolic and nuclear proteins (ubiquitin-proteasome pathway), and another for proteins found in or as a component of major intracellular structures such as vesicles and organelles (lysosomal proteolysis) [35].

Ammonia is a product of protein and AA metabolism which can be toxic for organelles [45-47]. Urea cycle is a critical pathway for detoxifying ammonia in mammals [46, 48]. There are 5 rate

limiting enzymes and two carriers in urea cycle including carbamoyl phosphate synthetase-I (CPS1), ornithine transcarboxylase (OTC), arginosuccinate synthetase (ASS), arginosuccinate lyase (ASL), arginase (ARG1), mitochondrial ornithine transporter 1 (ORNT1), and the mitochondrial aspartate/glutamate transporter (citrin) [47, 49, 50]. The initial step of urea cycle begins with the transfer of ammonia to a phosphorylated molecule of bicarbonate by carbamoyl phosphate synthase [47, 51]. The synthesis of citrulline from carbamoyl phosphate and ornithine is catalyzed by OTC [52]. ASS converts citrulline to arginosuccinate, the precursor of arginine [53]. ASL converts arginosuccinate to fumarate and arginine [54] and ARG catalyzes the conversion of arginine into ornithine and urea [55]. Deficiency in urea cycle enzymes or transporters results in urinary cycle disorders [50]. Nitrogen balance in the body is regulated by urea generation [56, 57]. Although mammalian tissues are unable to further utilize urea, urea significantly limits ureagenesis as a form of negative feedback regulation [58].

2.4. Tryptophan: effects on glucose, lipid, and protein metabolism

2.4.1. Tryptophan structure

Tryptophan is the sole AA found in proteins derived from indole, a bicyclic ring constituted from a benzene and a pyrrole group which is connected to an α-carbon by a -CH_2-group. In comparison to other AA, Trp has the highest level of hydrophobic properties due to the indole ring present in its chemical composition. Tryptophan is assumed to have the simplest structure among all potential indole AA, which leads to retain in protein of living organisms. Tryptophan has the highest carbon atoms (C11); hence the addition of further carbon atoms is not required. The benefit of preserved indole in biological chemistry comes from the ability to use its C11 skeleton in metabolism or from the ability to use it as a -R residue in proteins and peptides to support and stabilize their structure [59]. Tryptophan -R groups must be present in specific domains for a protein to be stable to the phospholipid bilayer, such as the transmembrane domains of membrane-bound proteins (Figure 2.1) [60]. Tryptophan residues in the AA sequence of small bioactive peptides can function as

melanocortin peptides, innate immune system defensive antimicrobial peptides, or endogenous anti-inflammatory or antiobesity peptides [61, 62].

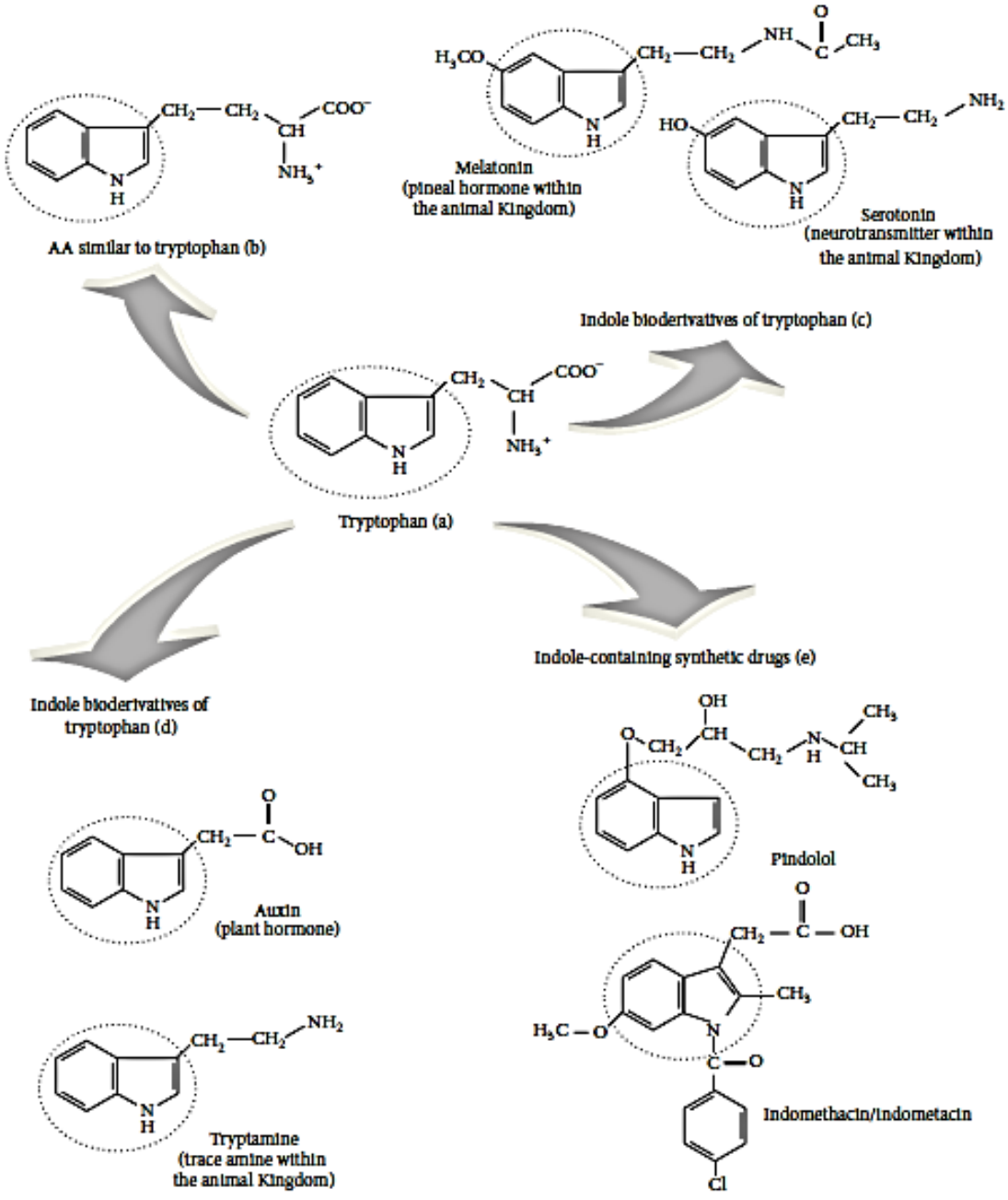


Figure 2.1. Tryptophan and other indole-containing compounds [59]

2.4.2. Tryptophan functions

As an essential AA, Trp is involved in numerous physiological functions [63]. Under health and disease conditions Trp and its metabolites have therapeutic benefits. In addition to being incorporated into body proteins, Trp serves as a precursor for the synthesis of serotonin, niacin CO2, and kynurenic and xanthurenic acids. Tryptophan controls immune response during infections, inflammations and pregnancy and known to influence mood disorders [63].

2.4.3. Tryptophan metabolism

After absorption, Trp can enter either to the protein metabolism and synthesis or multiple metabolic pathways. Tryptophan metabolism can be split into two primary pathways. First, pathways that maintain the integrity of the indole ring to produce messengers such as serotonin (5-hydroxytryptamine (5-HT)), N-acetyl-5-HT, and melatonin as well as the trace amine tryptamine and derivatives. Roughly 3-10 % of Trp goes to this pathway. Second, pathways that breaks the indole ring to produce the kynurenine path, kynurenines, nicotinic acid, and the synthesis of nicotinamide adenine dinucleotide (NAD+). About 90% of Trp goes to this pathway (Figure 2.2) [59].

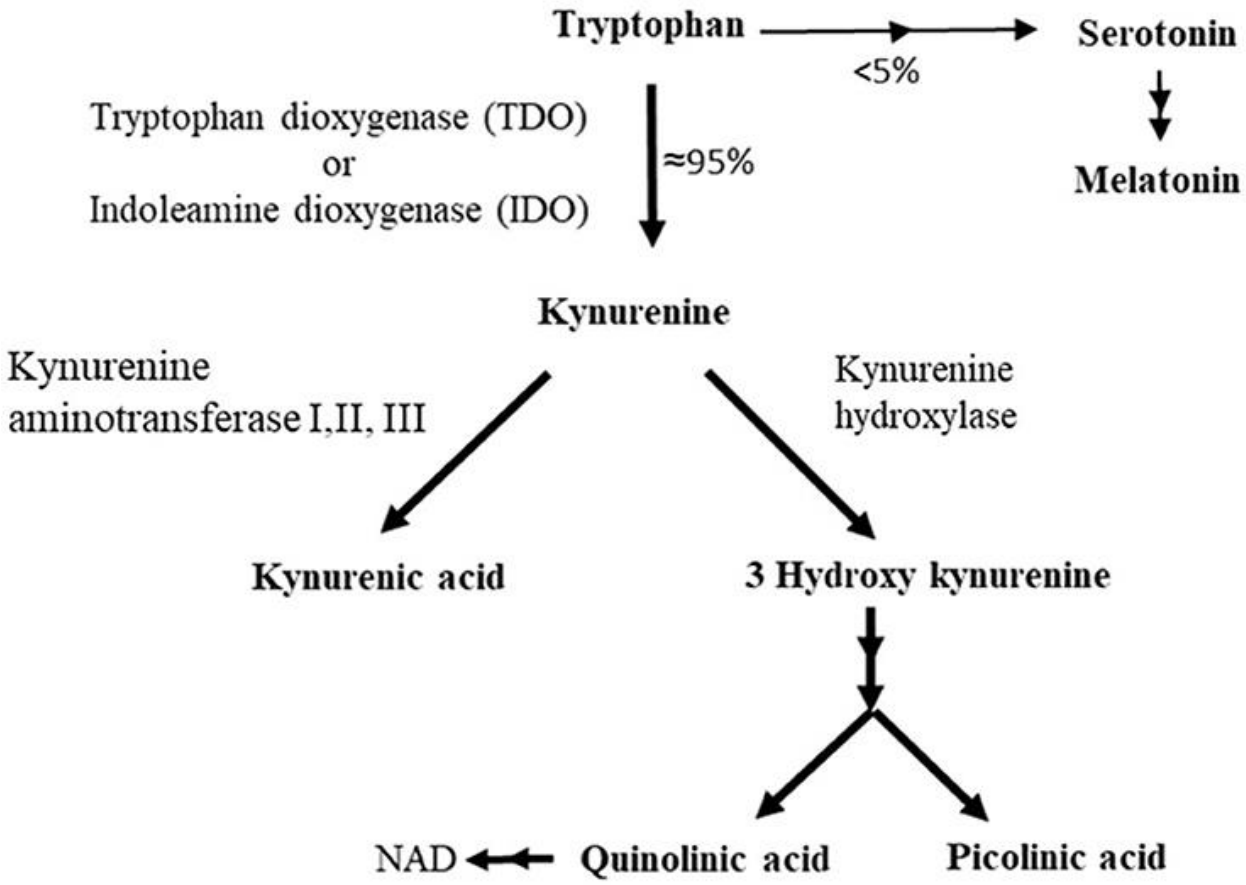


Figure 2.2. Tryptophan metabolic routes in humans [64]

Serotonin, a Trp derivatives, is an essential neuromediator that controls gastrointestinal processes, mood, appetite, and hemodynamics. The conversion of Trp to serotonin includes the synthesis of 5-hydroxytryptophan by the tryptophan hydroxylase and decarboxylation of 5-hydroxytryptophan into serotonin using vitamin B6 as a cofactor. It is important to highlight that tryptophan hydroxylase exists in two distinct isoforms, tryptophan hydroxylase-1 in enterochromaffin cells and tryptophan hydroxylase-2 in neurons (Figure 2.3) [63].

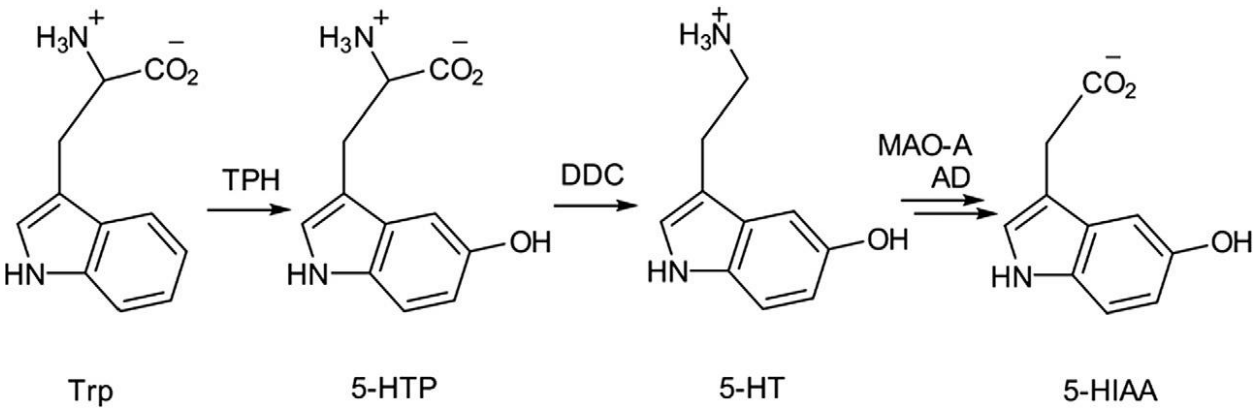


Figure 2.3. Tryptophan metabolism: serotonin pathway [64]

The kynurenine (KNY) pathway, which is a key catabolic pathway for Trp, is crucial for the production of nicotinic acid (niacin), quinolinic acid, kynurenic acid, and xanthurenic acid [65]. The initial phase of Trp breakdown, the synthesis of N-formyl kynurenine, is catalyzed by tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenases (IDO-1 and IDO-2). These enzymes differ from one another in their tissue location, structure, substrate selectivity, cofactor need, and activity. They also differ in how they catalyze the identical oxidation reaction involving the breakdown of the pyrrole ring to generate N-formylkynurenine [63, 64]. TDO is primarily found in the liver, while it can also be expressed in the brain [66, 67]. This enzyme is stimulated by its substrate Trp, glucocorticoids and the cofactor heme [68-71]. Further, IDO-1 is present in many tissues, but IDO-2 is mostly found in the kidney, liver, and brain [64]. Pro-inflammatory cytokines, interferon, bacteria lipopolysaccharides, viruses, and tumor cells all control IDO enzymes [72-74]. Moreover, N-formylkynurenine formamidase transforms N-formyl kynurenine to KYN once it has formed. The enzymes KYN aminotransferase and kynureninase transform KYN into kynurenic acid and anthranilic acid, respectively. In addition, kynurenine 3-monooxygenase converts KYN into 3-hydroxykynurenine, which is then transaminated to xanthurenic acid. Then kynureninase converts xanthurenic acid to to 3-hydroxyanthranilic acid. Moreover, nicotinic acid and quinolinic acid can be produced from 3-hydroxyanthranilic acid via 3-hydroxyanthranilate 3,4-dioxygenase.

Then quinolinic acid is converted to picolinic acid. Further, nicotinic acid, the precursor for the NAD coenzyme, is produced through decarboxylation of quinolinic acid (Figure 2.4) [64].

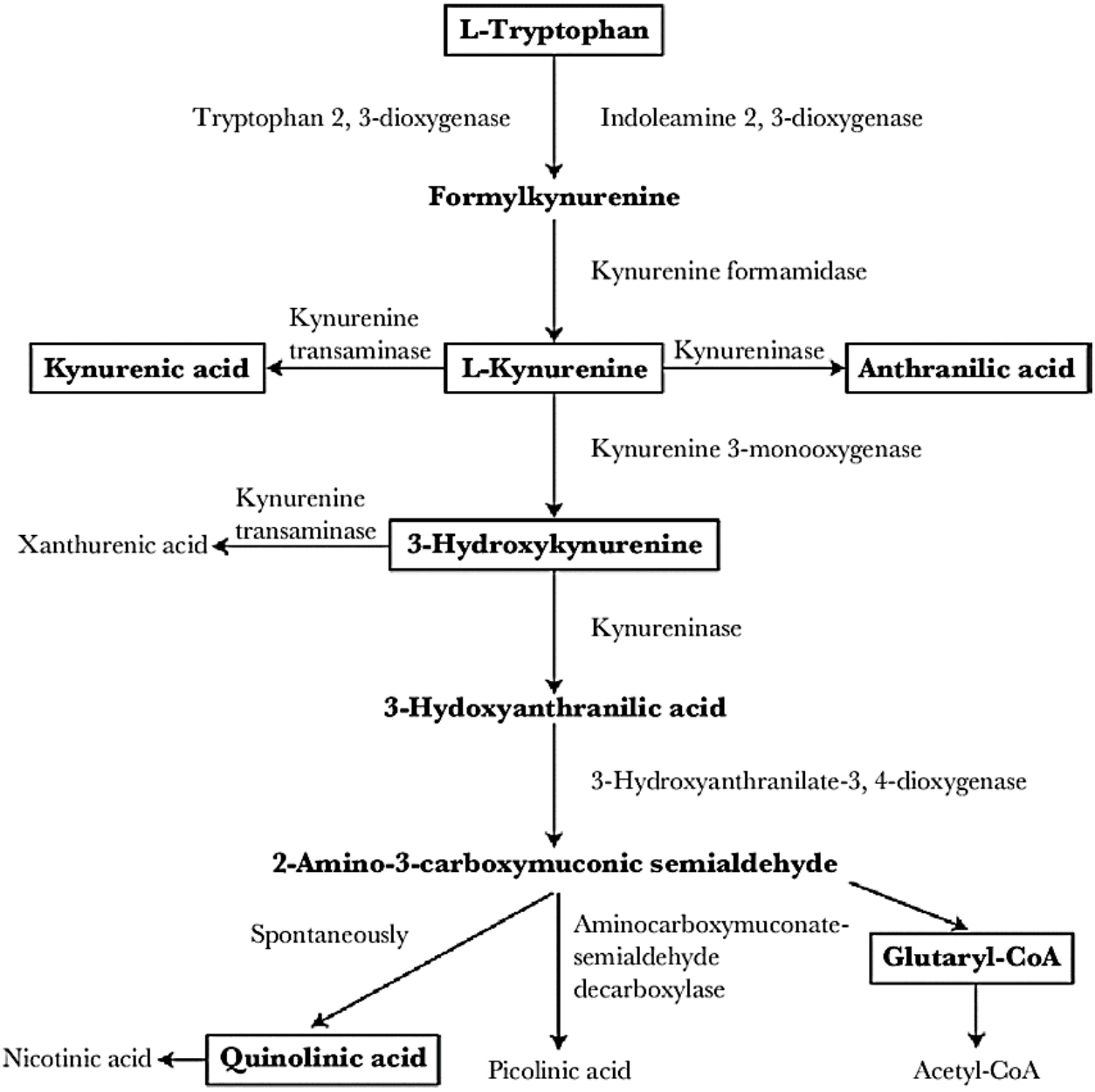


Figure 2.4.Tryptophan metabolism: kynurenine pathway [64]

2.4.4. Effect of Trp on lipid metabolism

Previous research demonstrated that liver lipid content considerably decreased when Trp was increased in the diet of growing broiler chicks suggestive of a potential link between Trp and lipid metabolism [75]. Intragastric administration of Trp to rats reduced the fat deposition, unsaturated lipids, LDL, VLDL, and triglycerides concentrations in the serum and increased the ratio of acetoacetate and hydroxybutyrate, the two main byproducts of fatty acid oxidation in the rat liver [76, 77]. Moreover, a study demonstrated that Trp degradation by indoleamine 2, 3-dioxygenase enhanced the fatty acids beta oxidation in CD4+ T cells of human mixed lymphocyte reactions by activating CPT1. In support of this view, malonyl-CoA is reduced by Trp which allosterically control CPT1 [78]. Further, the other study showed that Trp metabolite, melatonin, prevents the oxidative stress resulting in lower hyperglycemia and hyperlipidemia during type 1 diabetes mellitus [79]. Further research is needed to better understand the molecular pathways for lipid metabolism regulated by Trp.

2.4.5. Effect of Trp on glucose metabolism

There are controversial data on the effect of Trp on glucose metabolism. Some studies reported that Trp might stimulate glucagon secretion which stimulates the glycogenolysis and gluconeogenesis [80]. In contrast, other studies have shown that Trp reduces the circulating glucose concentration via different pathways [81]. Tryptophan may reduce the glucose absorption in small intestine through reducing the protein abundance of SGLT1 [81]. Further, tryptamine, a Trp metabolite, was found to increase the rate of glucose uptake in adipocytes by stimulation of insulin [81]. In addition, tryptamine-induced insulin may result in hypoglycemia in normal mice [82]. Another Trp metabolites is melatonin which can enhance the insulin action and pancreatic beta cells functions [83]. In rats, removing the pineal gland increased glucose [84] and glucagon and lowered insulin levels to baseline level [85]. It has been demonstrated that pinealectomized rats develop hepatic insulin resistance and increase gluconeogenesis and PEPCK expression [86]. An insulin-resistant mouse model demonstrated an enhancement in glucose metabolism after treatment with melatonin

[87]. Moreover, serotonin, has been found to increase glucose absorption in skeletal muscle and liver, followed by glycogen synthase, by activating cyclin dependent kinase 5 [88-90]. Niacin is another Trp metabolite which is important for glucose metabolism [91]. According to several studies, Trp administration in normal and adrenalectomized rats reduced PEPCK activity [92]. Of note, some studies showed the paradoxical effect of Trp on PEPCK activity in rat and mice [93, 94]. Another process that Trp may have a role, is glycolysis which glucose is converted to pyruvate by producing energy. Tryptophan either had no effect on GCK expression in rats [95] or decreased GCK expression in blunt snout bream [96].

2.4.6. Effect of Trp on protein metabolism

A particular aminoacyl-tRNA synthetase is activated during the production of proteins and uses ATP hydrolysis to transfer Trp to the appropriate tRNA. This enzyme has two isoforms in the cytoplasm and mitochondria [97]. Tryptophanyl-tRNA synthetases isoforms serve important roles in protein synthesis, innate immunity control, angiogenesis, and IFN- signaling. They are formed through alternative splicing and proteolytic cleavage [98]. Regarding protein degradation, plasma nitrogen is used as criteria for the protein utilization efficiency. Plasma urea nitrogen in pigs fed with dietary Trp was decreased which indicates that protein degradation was reduced or utilization of nitrogen was improved [99]. Consistent with other studies, increased dietary Trp reduced the excreted nitrogen and urinary nitrogen, and increased the protein deposition in pigs fed with Trp-limiting diets [100]. The reduction in plasma urea nitrogen might be due to effect of Trp on reduction of gluconeogenesis via PEPCK. It has been shown that Trp reduced both the activity and mRNA expression of PEPCK resulting in an increased oxaloacetate and other metabolites of citric acid cycle. Therefore, transamination of glutamate into alpha ketoglutarate was promoted which resulted in using ammonia and reducing plasma nitrogen. On the other hand, local CO2 production in the liver was reduced as a result of partial suppression of oxaloacetate decarboxylation, leading to carbamoyl phosphate accumulation. Therefore, citrulline formation and urea production

decreased. Moreover, Trp metabolites could reduce the rate of oxidative phosphorylation, in which causes a lower carbamoyl phosphate synthetase activity [101].

2.5. Isoleucine: effects on glucose, lipid, and protein metabolism

2.5.1. Isoleucine structure

The unsubstituted aliphatic chain with the branched alkyl group in BCAA makes them among the most hydrophobic AA [102]. Leucine and Ile both have isobutyl groups as their side chains, and their branching occurs on the γ- and β-carbons, respectively (Figure 2.5) [103]. When a side chain is aliphatic, it solely has hydrogen and carbon atoms. In contrast to other AA, which only have one non-hydrogen substituent connected to their Cβ carbon, Ile and Val have two of them which means these AA preferred to lie in β-sheets rather than α-helical conformation [104].

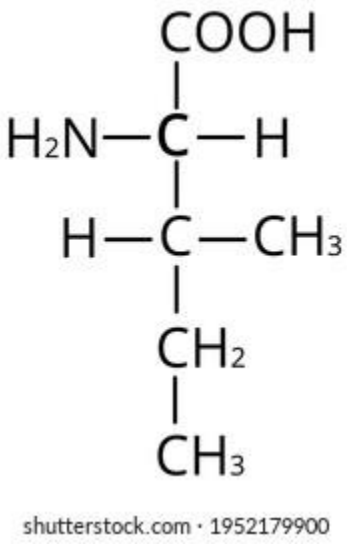


Figure 2.5. Isoleucine chemical structure from shutterstock.com.

Isoleucine prefers to be in the hydrophobic cores of proteins because of its hydrophobic properties. Moreover, BCAA are essential for globular shape of proteins and the interaction of phospholipid bilayers with transmembrane domains [102].

2.5.2. Isoleucine functions

Isoleucine is considered as both glucogenic and ketogenic [105] and plays a crucial role in physiological processes, including growth, immunity, protein metabolism, fatty acid metabolism, and glucose transport. The immune system, including immune cells, organs, and reactive chemicals, can be enhanced by Ile. A host's innate and adaptive immunity can be regulated by defense peptides, such as β-defensins, which their expression can be regulated by Ile [106].

2.5.3. Isoleucine metabolism

The majority of BCAA are readily available for metabolism in skeletal muscle and other tissues, except liver. However, after being transformed into α-ketoacids in other tissues, BCAA ketoacids enter into the liver for further metabolism [107]. BCAA are catabolized into branched-chain α-ketoacids by the enzyme branched-chain aminotransferase (BCAT). Isoleucine is converted into α-keto-β-methylvalerate by the removal of its amino group by BCAT. In the following step, branched-chain α-ketoacid dehydrogenase (BCKD), decarboxylates α-keto-β-methylvalerate which after series of reactions, acetyl-CoA and succinyl-CoA formed to fuel TCA cycle or other metabolic pathway (Figure 2.6) [2, 108].

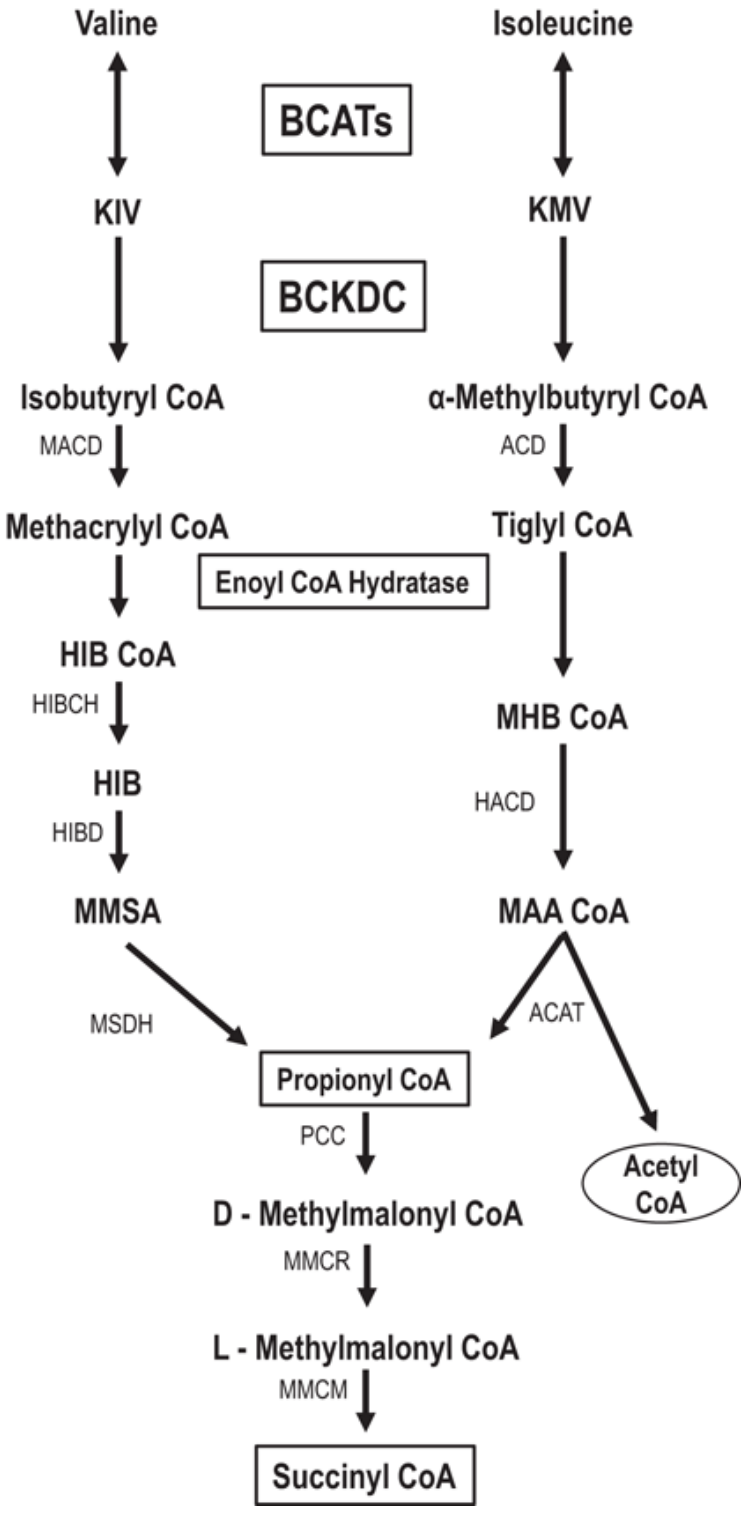


Figure 2.6. Isoleucine and Val catabolism pathway [109]

2.5.4. Effect of Ile on lipid metabolism

Isoleucine (α-amino-β-methylvaleric acid) is a multifunctioning BCAA which plays a role in lipid metabolism. Isoleucine affects adipose tissue hormones such as leptin and adiponectin, leading to reduced adiposity in white adipose tissue. Isoleucine can decrease leptin and increase adiponectin hormones in obese mice [110]. It has been shown that high level of leptin during high fat diet-induced obesity is decreased by dietary Ile in mice [111]. Moreover, Ile can reduce triglyceride concentration in skeletal muscle and liver presumably via the action of adiponectin [111]. In diet-induced obese mice, Ile reduces adiposity via mobilization of lipid without affecting the food intake [111]. It has been reported that Ile has no effect on the expression of brown adipose tissue uncoupling protein 1 (UCP-1), suggesting that reduction in adiposity by Ile may be regulated by mechanisms other than brown adipose tissue energy expenditure [112, 113].

Liver and skeletal muscle are the main organs for fatty acid oxidation. Free fatty acid uptake into the tissues is dependent upon on the level of key regulators such as PPAR-α, UCP-2, UCP-3 and fatty acid translocase (FAT)/ CD36. PPAR-α controls the expression of several genes involved in lipid and lipoprotein metabolism. UCP-2 and UCP-3 are proteins located in mitochondria that may regulate energy expenditure by uncoupling respiration from ATP production. As a long chain fatty acid transporter in mitochondria, FAT/CD36, enhanced fatty acid oxidation ability. Isoleucine has been shown to increase PPAR-α expression in liver and skeletal muscle of mice which enhances the fatty acid oxidation and prevents the development of obesity and insulin resistance. It appears that Ile stimulates PPAR-α activation and expression, leading to a lower adiposity. The effect of Ile on the increased expression of hepatic UCP-2 and muscle UCP-3 is regulated by PPAR-α. It has been demonstrated that UCP-2 and UCP-3 regulate fatty acid oxidation. Moreover, Ile increases the FAT/CD36 expression in mice skeletal muscle and liver indicating that Ile might accelerate fatty acids uptake. Therefore, Ile promotes free fatty acid uptake and oxidation in the liver and skeletal muscle via PPAR-α and UCPs at the same time [111].

In hybrid catfish, dietary Ile alters the fatty acid and amino acid composition of the muscle by regulating the lipid metabolism and autophagy [114]. Some studies mentioned that Ile plays a role in lipogenesis in Murine 3T3-L1 pre-adipocytes [115]. Extra dietary Ile increased the intramuscular fat deposition and monounsaturated fatty acid synthesis in skeletal muscle via upregulation of lipogenic enzymes such as ACC in pigs [116]. Further research on the effect of Ile on lipid metabolism and the underlying mechanisms is required.

2.5.5. Effect of Ile on glucose metabolism

Isoleucine has been shown to improve glucose utilization and consumption in animals and humans. The fact that Ile stimulates glucose uptake without requiring insulin suggests that phosphoinositide 3-kinase and protein kinase C, but not mTOR, are involved in Ile-mediated glucose uptake enhancement in muscle cells [117]. Based on previous studies, Ile reduced the plasma glucose concentration even greater than Leu or Val in both animals and humans which shows hypoglycemic effect of Ile [118]. Moreover, Ile decreased the AMP content and the AMP to ATP ratio in rat skeletal muscle, indicating that Ile improves the ATP availability in skeletal muscle and thus enhances the cellular energy state. In addition, glucose oxidation was increased in Ile-administered rats which had no effect on glycogen synthesis, implying that blood glucose levels were decreased [118]. Of note, Ile improves glucose uptake mainly in skeletal muscle rather than liver or adipose tissue, indicating that skeletal muscle is the primary organ involved in Ile-induced hypoglycemia mediated by GLUT-1 and GLUT-4 [119]. Further, Ile reduced the mRNA expression and activity of PEPCK and glucose-6-phosphatase, two gluconeogenic rate-limiting enzymes that are inhibited under insulin-free conditions. Isoleucine can prevent glucose production by inhibiting alanine transportation, a neutral glucogenic substrate in liver, due to having same transporter [118].

2.5.6. Effect of Ile on protein metabolism

Several studies have shown that dietary Ile supplementation lowers the plasma urea concentration and plasma urea nitrogen. For example, in pigs fed with an Ile-deficient diet, supplementation of Ile reduced the plasma urea nitrogen levels [120]. Another study in pigs found that increasing dietary Ile level reduced the plasma urea and postulated that Ile utilization and protein synthesis were improved [121]. Furthermore, higher dietary Ile has been demonstrated to raise plasma concentrations of ammonia, citrulline, and ornithine. The rise in ammonia was related to increased deamination of AA in the liver or a decrease in carbamoyl phosphate production [122]. An increase in plasma citrulline and a decrease in plasma ornithine, which both are key substrates of the urea cycle, lead to a reduction in ureagenesis [122]. In support of this notion, Ile and Val catabolism generate propionyl-CoA which is a competitive substrate of N-acetylglutamate synthetase to form N-propionylglutamate rather than N-acetylglutamate. N-propionylglutamate acts as a poor activator of carbamylphosphate synthetase which converts ammonia to carbamyl phosphate [123-125].

2.6. Valine: effects on glucose, lipid, and protein metabolism

2.6.1. Valine structure

Valine or α-aminoisovaleric acid is classified among the most hydrophobic AA due to the unsubstituted aliphatic chain with the branched alkyl group [28, 102]. Valine specifically has a side chain of isopropyl group (Figure 2.7) [2, 28, 103].

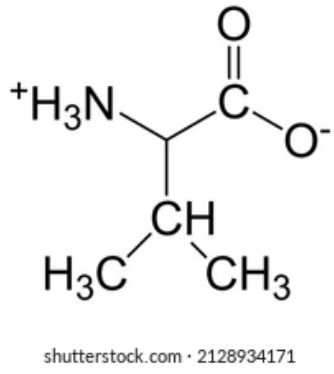


Figure 2.7. Valine chemical structure from shutterstock.com.

2.6.2. Valine functions

Valine is a glucogenic AA [105] that contributes to variety of physiological and metabolic processes, as well as the growth of animals [126-128]. Valine has been regarded as one of the limiting AA in poultry and pigs and it can enhance the lactation of breeding animals [27, 129].

Neither mTOR phosphorylation nor muscle protein turnover are affected by the Ile and Val [130, 131]. Moreover, Val has been shown to improve the host immunity and stimulate the macrophage phagocytosis [132].

2.6.3. Valine metabolism

In the first step of Val metabolism, BCAT converts Val into α-ketoisovalerate. Then, after a series of reactions, succinyl-CoA as the final product enters to the TCA cycle (Figure 2.6) [2, 108].

2.6.4. Effect of Val on lipid metabolism

According to the previous studies Val has strong influence on lipid metabolism [133-136]. It has been reported that fat synthesis is affected by plasma Val concentrations in rats and gilts [136, 137]. A study showed that Val elevated the expression and activity of ACC, FAS, fatty acid binding protein 3, and diacylglycerol O-acyltransferase 1 in porcine mammary epithelial cells, indicating an increased rate of lipid synthesis [133]. While Val deprivation reduced fat loss in mice [135], others showed that Val supplementation into the drinking water increased fat deposition and serum triglyceride and worsened the glucose and insulin tolerance [138]. Based on metabolomic analysis, the plasma level of polyunsaturated fatty acids was reduced in rats fed with diets supplemented with Val [139, 140]. This might be due to stimulation of insulin secretion via the lipoxygenase pathway [138]. Moreover, supplemented Val increased the PPARβ mRNA transcript, which could mitigate the hepatic steatosis by accelerating fatty acid oxidation and increasing the mRNA expression of CPT1α in liver [138, 141]. Further, Val suppresses fibroblast growth factor 21 (FGF21), which can stimulate fatty acid oxidation, improve insulin resistance and steatosis in obese mice and activate the secretion and expression of adiponectin [142-145]. In view of the foregoing, it was speculated that Val supplementation downregulates FGF21-adiponectin axis [138]. Additionally, dietary supplementation of Val increased the odd chain fatty acid levels in liver and blood of rats through alpha oxidation (by activation of PPARα). Valine catabolism supplies

propionyl-CoA which plays a role in de novo lipogenesis [146]. It has been shown that reduced incidence of type-2-diabetes mellitus is linked with increased levels of blood odd-chain fatty acids [147]. Moreover, another Val catabolite, 3-hydroxyisobutyrate (3-HIB), enhances fatty acids uptake in muscle *in vivo* and promotes lipid deposition in muscle, resulting in insulin resistance in mice [148]. Further investigation is required to determine the effect of Val on lipid metabolism and the mechanisms involved.

2.6.5. Effect of Val on glucose metabolism

The plasma glucose was increased by oral administration of Val in rats suggestive of a role for Val in glyconeogenesis in liver [117]. Valine, on the other hand, was reported to have no effects on glucose uptake in skeletal muscle and glycolysis, although it did increase the glycogen synthesis in rats [117]. Intrahypothalamic infusion of Val in rats reduced the blood glucose which presumably occurs through lowering hepatic glucose synthesis during a euglycemic pancreatic clamp [149]. It has been demonstrated that circulating Val levels might be a unique biomarker for type 2 diabetes and targeting Val metabolism might be a therapeutic strategy for type 2 diabetes and high-fat diet fed mice [150]. Val has also been shown to stimulate the secretion of glucagon,[151]. Further studies are required on the effect Val on glucose metabolism.

2.6.6. Effect of Val on protein metabolism

Among BCAA, Val has the least effect on phosphorylation of 4E-BP1 or S6K1 [152]. Besides, the role of Val metabolite, propionyl-CoA, in decreasing the carbamoyl phosphate [123, 125], it has been demonstrated that norvaline, an unbranched Val isomer, can inhibit ornithine transcarbamylase [153]. Further, norvaline with ammonia infusion reduced the ureagenesis and stimulated glycogen synthesis. It has been shown that norvaline can activate glycogen synthesis in the presence of ammonia [154, 155]. In contrast, a study showed that intraperitoneal injection of

Val to rat, increased the ornithine content through inhibition of ornithine-keto acid transaminase resulted in urea cycle activation [156].

2.7. Pig as a model for nutrients metabolism studies

In both healthy and diseased conditions, rodents have been extensively employed as models for human nutrition, physiology, and pathophysiology; however, rodents in most cases fall short of accurately simulating human conditions [157]. Pigs more closely resemble humans in terms of their biochemistry, cell biology, anatomy, physiology, and pathophysiology as compared to rodents [157]. The pig exhibits a significant degree of chromosomal and DNA similarities with human beings [158]. Pigs are monogastric omnivores, and as such, their nutritional needs, digestion, and nutrient absorption physiology are quite similar to that of humans [159]. Pigs have served as a research model for studying human energy metabolism and nutritional sciences and metabolic syndromes such as obesity [160]. Additionally, the pig's metabolic and cardiovascular systems, as well as its roughly equivalent organ sizes, are similar to human [160]. Adipocytes or stromal vascular cells can be subjected to several assays in pigs due to adequate size of their fat depots [160]. The subcutaneous fat of pigs is similar in anatomy to humans, which is the largest fat depot in both species [161]. Pigs' pancreas is similar to the human pancreas in size, shape, and blood circulation, and pig has become a popular diabetes model since insulin is secreted by the pancreatic islet cells [162]. Using gene editing and transgenic procedures for generating pigs with interrupted insulin signaling, pigs have served as essential research models for understanding the development of diabetes [163, 164]. The Gottingen, Yucatan, and Ossabaw swine breeds have been employed frequently for research on obesity and cardiovascular diseases, although the Ossabaw breed seems to be a particularly suitable model [160]. Importantly, it has been demonstrated that pigs can be used as model for amino acid metabolism of humans [165]. Further, pigs have been suggested as suitable animal models for studying dietary modulation of the human gut microbiota [166, 167].

2.8. References

1. MacFarlane, N.G. Digestion and absorption. *Anaesth. Intensive Care Med.* **2018**, *19*, 125–127. DOI: 10.1016/j.mpaic.2018.01.001.

2. Gropper, S.S.; Smith, J.L., *Advanced nutrition and human metabolism*. 2012, Boston, MA: Cengage Learning.

3. Jackson, A.D.; McLaughlin, J. Digestion and absorption. *Surgery* **2009**, *27*, 231–236. DOI: 10.1016/j.mpsur.2009.03.003

4. Verger, R.; Rietsch, J.; Desnuelle, P. Effects of colipase on hydrolysis of monomolecular films by lipase. *J. Biol. Chem.* **1977**, *252*, 4319–4325. DOI: 10.1016/S0021-9258(17)40267-5

5. Brockerhoff, H. On the function of bile salts and proteins as cofactors of lipase. *J. Biol. Chem.* **1971**, *246*, 5828–5831. DOI: 0.1016/S0021-9258(18)61880-0

6. Hernell, O.; Staggers, J.E.; Carey, M.C. Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. *Biochemistry* **1990**, *29*, 2041–2056. DOI: 10.1021/bi00460a012

7. Hinsberger, A.; Sandhu, B. Digestion and absorption. *Current Paediatrics* **2004**, *14*, 605–611. DOI: 10.1016/j.cupe.2004.08.004

8. Yen, C.-L.E.; Nelson, D.W.; Yen, M.-I. Intestinal triacylglycerol synthesis in fat absorption and systemic energy metabolism. *J. Lipid Res.* **2015**, *56*, 489–501. DOI: 10.1194/jlr.R052902.

9. Kersten, S. Physiological regulation of lipoprotein lipase. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* **2014**, *1841*, 919–933. DOI: 10.1016/j.bbalip.2014.03.013

10. Davies, B.S.; Beigneux, A.P.; Barnes II, R.H.; Tu, Y.; Gin, P.; Weinstein, M.M.; Nobumori, C.; Nyrén, R.; Goldberg, I.; Olivecrona, G. GPIHBP1 is responsible for the entry of lipoprotein lipase into capillaries. *Cell Metab.* **2010**, *12*, 42–52. DOI: 10.1016/j.cmet.2010.04.016.

11. Beigneux, A.P.; Davies, B.S.; Gin, P.; Weinstein, M.M.; Farber, E.; Qiao, X.; Peale, F.; Bunting, S.; Walzem, R.L.; Wong, J.S. Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 plays a critical role in the lipolytic processing of chylomicrons. *Cell Metab.* **2007**, *5*, 279–291. DOI: 10.1016/j.cmet.2007.02.002.

12. Korn, E.D. Clearing factor, a heparin-activated lipoprotein lipase: I. Isolation and characterization of the enzyme from normal rat heart. *J. Biol. Chem.* **1955**, *215*, 1–14. DOI: 10.1016/S0021-9258(18)66011-9.

13. Kersten, S. Mechanisms of nutritional and hormonal regulation of lipogenesis. *EMBO reports* **2001**, *2*, 282–286. DOI: 10.1093/embo-reports/kve071

14. Horton, J.D.; Goldstein, J.L.; Brown, M.S. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Investig.* **2002**, *109*, 1125–1131. DOI: 10.1172/JCI15593

15. Schoonjans, K.; Staels, B.; Auwerx, J. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J. Lipid Res.* **1996**, *37*, 907–925. DOI: 10.1016/S0022-2275(20)42003-6

16. Lieberman, M.; Marks, A.D., *Marks' basic medical biochemistry: a clinical approach*. 2009: Lippincott Williams and Wilkins.

17. Binder, H.J.; Reuben, A. Nutrient digestion and absorption. *Medical physiology: a cellular molecular approach. Philadelphia: Saunders Elsevier* **2009**, 971.

18. Adeva-Andany, M.M.; Pérez-Felpete, N.; Fernández-Fernández, C.; Donapetry-García, C.; Pazos-García, C. Liver glucose metabolism in humans. *Biosci. Rep.* **2016**, *36*, DOI: 10.1042/BSR20160385.

19. Yeo, R.; Sawdon, M. Hormonal control of metabolism: regulation of plasma glucose. *Anaesth. Intensive Care Med.* **2013**, *14*, 296–300. DOI: 10.1016/j.mpaic.2013.04.009.

20. Giugliano, D.; Ceriello, A.; Esposito, K. Glucose metabolism and hyperglycemia. *Am. J. Clin. Nutr.* **2008**, *87*, 217S–222S.

21. Ang, M.; Linn, T. Comparison of the effects of slowly and rapidly absorbed carbohydrates on postprandial glucose metabolism in type 2 diabetes mellitus patients: a randomized trial. *Am. J. Clin. Nutr.* **2014**, *100*, 1059–1068.

22. Aronoff, S.L.; Berkowitz, K.; Shreiner, B.; Want, L. Glucose metabolism and regulation: beyond insulin and glucagon. *Diabetes Spectr.* **2004**, *17*, 183–190. DOI: 10.2337/diaspect.17.3.183.

23. Iynedjian, P.; Marie, S.; Gjinovci, A.; Genin, B.; Deng, S.-P.; Buhler, L.; Morel, P.; Mentha, G. Glucokinase and cytosolic phosphoenolpyruvate carboxykinase (GTP) in the human liver. Regulation of gene expression in cultured hepatocytes. *J. Clin. Investig.* **1995**, *95*, 1966–1973. DOI: 10.1172/JCI117880.

24. Woerle, H.J.; Meyer, C.; Dostou, J.M.; Gosmanov, N.R.; Islam, N.; Popa, E.; Wittlin, S.D.; Welle, S.L.; Gerich, J.E. Pathways for glucose disposal after meal ingestion in humans. *Am. J. Physiol. Endocrinol. Metab.* **2003**, *284*, E716–E725. DOI: 10.1152/ajpendo.00365.2002.

25. Chandramouli, V.; Ekberg, K.; Schumann, W.C.; Kalhan, S.C.; Wahren, J.; Landau, B.R. Quantifying gluconeogenesis during fasting. *Am. J. Physiol. Endocrinol. Metab.* **1997**, *273*, E1209–E1215. DOI: 10.1152/ajpendo.1997.273.6.E1209

26. Paksu, M.S.; Kalkan, G.; Asilioglu, N.; Paksu, S.; Dinler, G. Gluconeogenesis defect presenting with resistant hyperglycemia and acidosis mimicking diabetic ketoacidosis. *Pediatr. Emerg. Care* **2011**, *27*, 1180–1181. DOI: 10.1097/PEC.0b013e31823b412d.

27. Wu, G. Amino acids: metabolism, functions, and nutrition. *Amino Acids* **2009**, *37*, 1–17. DOI: 10.1007/s00726-009-0269-0.

28. Wu, G., *Amino acids: biochemistry and nutrition*. 2021: CRC Press. DOI: 10.1201/9781003092742

29. Takikawa, H.; Yamanaka, M. Mechanism of amino acid transport at the liver plasma membrane. *Nihon Rinsho* **1992**, *50*, 1475–1479.

30. Hou, Y.; Hu, S.; Li, X.; He, W.; Wu, G. Amino acid metabolism in the liver: nutritional and physiological significance. *Amino Acids in Nutrition Health* **2020**, 21–37. DOI: 10.1007/978-3-030-45328-2_2.

31. Wheatley, S.M.; El-Kadi, S.W.; Suryawan, A.; Boutry, C.; Orellana, R.A.; Nguyen, H.V.; Davis, S.R.; Davis, T.A. Protein synthesis in skeletal muscle of neonatal pigs is enhanced by administration of β-hydroxy-β-methylbutyrate. *Am. J Physiol. Endocrinol. Metab.* **2014**, *306*, E91–E99. DOI: 10.1152/ajpendo.00500.2013.

32. Brosnan, J.T. Interorgan amino acid transport and its regulation. *J. Nutr.* **2003**, *133*, 2068S–2072S. DOI: 10.1093/jn/133.6.2068S.

33. Gilbreath, K.R.; Bazer, F.W.; Satterfield, M.C.; Wu, G. Amino acid nutrition and reproductive performance in ruminants. In *Amino Acids in Nutrition and Health*, 2021, 43–61. DOI: 10.1007/978-3-030-54462-1_4.

34. Li, X.; Zheng, S.; Wu, G. Nutrition and functions of amino acids in fish. In *Amino Acids in Nutrition and Health*, 2021, 133–168. DOI: 10.1007/978-3-030-54462-1_8.

35. Rothman, S. How is the balance between protein synthesis and degradation achieved? *Theor. Biol. Medical Model.* **2010**, *7,* 1–11. DOI: 10.1186/1742-4682-7-25.

36. Gingras, A.-C.; Raught, B.; Sonenberg, N. eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Annu. Rev. Biochem.* **1999**, *68*, 913–963.

37. Wang, X.; Proud, C.G. The mTOR pathway in the control of protein synthesis. *Physiology* **2006**, *21,* 362–369. DOI: 10.1152/physiol.00024.2006.

38. Proud, C. Control of the elongation phase of protein synthesis. *Cold Spring Harbor Monogr. Ser.* **2000**, *39*, 719–740.

39. Bazer, F.W.; Song, G.; Kim, J.; Erikson, D.W.; Johnson, G.A.; Burghardt, R.C.; Gao, H.; Satterfield, M.C.; Spencer, T.E.; Wu, G. Mechanistic mammalian target of rapamycin (MTOR) cell signaling: effects of select nutrients and secreted phosphoprotein 1 on development of mammalian conceptuses. *Mol. Cell. Endocrinol.* **2012**, *354,* 22–33. DOI: 10.1016/j.mce.2011.08.026.

40. Wu, G.; Bazer, F.W.; Dai, Z.; Li, D.; Wang, J.; Wu, Z. Amino acid nutrition in animals: protein synthesis and beyond. *Annu Rev Anim Biosci* **2014**, *2,* 387–417. DOI: 10.1146/annurev-animal-022513-114113.

41. Morita, M.; Gravel, S.-P.; Hulea, L.; Larsson, O.; Pollak, M.; St-Pierre, J.; Topisirovic, I. mTOR coordinates protein synthesis, mitochondrial activity and proliferation. *Cell cycle* **2015**, *14,* 473–480. DOI: 10.4161/15384101.2014.991572.

42. Sarbassov, D.D.; Guertin, D.A.; Ali, S.M.; Sabatini, D.M. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* **2005**, *307*, 1098–1101. DOI: 10.1126/science.1106148.

43. Horman, S.; Browne, G.J.; Krause, U.; Patel, J.V.; Vertommen, D.; Bertrand, L.; Lavoinne, A.; Hue, L.; Proud, C.G.; Rider, M.H. Activation of AMP-activated protein kinase leads to the phosphorylation of elongation factor 2 and an inhibition of protein synthesis. *Curr. Biol.* **2002**, *12*, 1419–1423. DOI: 10.1016/S0960-9822(02)01077-1.

44. Browne, G.J.; Finn, S.G.; Proud, C.G. Stimulation of the AMP-activated protein kinase leads to activation of eukaryotic elongation factor 2 kinase and to its phosphorylation at a novel site, serine 398. *J. Biol. Chem.* **2004**, *279*, 12220–12231. DOI: 10.1074/jbc.M309773200.

45. Dimski, D.S. Ammonia metabolism and the urea cycle: function and clinical implications. *J. Vet. Intern. Med.* **1994**, *8*, 73–78. DOI: 10.1111/j.1939-1676.1994.tb03201.x.

46. Katunuma, N.; Okada, M.; Nishii, Y.J.A.i.e.r. Regulation of the urea cycle and TCA cycle by ammonia. **1966**, *4*, 317-335. DOI: 10.1016/0065-2571(66)90025-2

47. Song, X.; Teng, L.; Zhao, X. Re-examining the origin and functions of the urea cycle. **2022**, DOI: 10.21203/rs.3.rs-1532969/v1.

48. Jackson, M.J.; Beaudet, A.L.; O'Brien, W.E. Mammalian urea cycle enzymes. *Annu. Rev. Genet.* **1986**, *20*, 431–464. DOI: 10.1146/annurev.ge.20.120186.002243

49. Morris Jr, a.S.M. Regulation of enzymes of the urea cycle and arginine metabolism. *Annu. Rev. Nutr.* **2002**, *22*, 87–105. DOI: 10.1146/annurev.nutr.22.110801.140547.

50. Bigot, A.; Tchan, M.C.; Thoreau, B.; Blasco, H.; Maillot, F. Liver involvement in urea cycle disorders: a review of the literature. *J. Inherited Metab. Dis.* **2017**, *40,* 757–769. DOI: 10.1007/s10545-017-0088-5.

51. de Cima, S.; Polo, L.M.; Díez-Fernández, C.; Martínez, A.I.; Cervera, J.; Fita, I.; Rubio, V. Structure of human carbamoyl phosphate synthetase: deciphering the on/off switch of human ureagenesis. *J Scientific reports* **2015**, *5,* 1–15. DOI: 10.1038/srep16950.

52. Couchet, M.; Breuillard, C.; Corne, C.; Rendu, J.; Morio, B.; Schlattner, U.; Moinard, C. Ornithine Transcarbamylase–From Structure to Metabolism: An Update. *Front. Physiol.* **2021**, *12,* DOI: 10.3389/fphys.2021.748249.

53. Haines, R.J.; Pendleton, L.C.; Eichler, D.C. Argininosuccinate synthase: at the center of arginine metabolism. *Int. J. Biochem. Mol.* **2011**, *2,* 8.

54. Cao, Y.L.; Li, G.L.; Wang, K.T.; Zhang, H.Y.; Li, L.F. Crystallization and preliminary X-ray analysis of argininosuccinate lyase from Streptococcus mutans. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* **2011**, *67,* 682–684. DOI: 10.1107/S1744309111011377.

55. Caldwell, R.B.; Toque, H.A.; Narayanan, S.P.; Caldwell, R.W. Arginase: an old enzyme with new tricks. *Trends Pharmacol. Sci.* **2015**, *36,* 395–405. DOI: 10.1016/j.tips.2015.03.006.

56. Young, V.R.; El-Khoury, A.E.; Raguso, C.A.; Forslund, A.H.; Hambraeus, L. Rates of urea production and hydrolysis and leucine oxidation change linearly over widely varying protein intakes in healthy adults. *J. Nutr.* **2000**, *130,* 761–766. DOI: 10.1093/jn/130.4.761.

57. Wang, H.; Ran, J.; Jiang, T. Urea. *Urea Transporters* **2014**, 7–29. DOI: 10.1007/978-94-017-9343-8_2.

58. Emmanuel. Autoregulation of urea cycle by urea in mammalian species. *Comp. Biochem. Physiol.* **1981**, *70*, 79–81. DOI: 10.1016/0300-9629(81)90398-4.

59. Palego, L.; Betti, L.; Rossi, A.; Giannaccini, G. Tryptophan biochemistry: structural, nutritional, metabolic, and medical aspects in humans. *J. Amino Acids* **2016**, *2016*, DOI: 10.1155/2016/8952520.

60. de Jesus, A.J.; Allen, T.W. The role of tryptophan side chains in membrane protein anchoring and hydrophobic mismatch. *Biochim. Biophys. Acta - Bioenerg.* **2013**, *1828*, 864–876. DOI: 10.1016/j.bbamem.2012.09.009.

61. Grieco, P.; Cai, M.; Mayorov, A.V.; Trivedi, D.; Hruby, V.J. Structure–activity studies of new melanocortin peptides containing an aromatic amino acid at the N-terminal position. *Peptides* **2006**, *27*, 472–481. DOI: 10.1016/j.peptides.2005.01.032.

62. Mojsoska, B.; Jenssen, H. Peptides and peptidomimetics for antimicrobial drug design. *Pharmaceuticals* **2015**, *8*, 366–415. DOI: 10.3390/ph8030366.

63. Floc'h, L.; Otten, W.; Merlot, E. Tryptophan metabolism, from nutrition to potential therapeutic applications. *Amino Acids* **2011**, *41*, 1195–1205. DOI: 10.1007/s00726-010-0752-7.

64. Comai, S.; Bertazzo, A.; Brughera, M.; Crotti, S. Tryptophan in health and disease. *Adv. Clin. Chem.* **2020**, *95*, 165–218. DOI: 10.1016/bs.acc.2019.08.005.

65. Badawy, A.A. Kynurenine pathway of tryptophan metabolism: regulatory and functional aspects. *Int. J. Tryptophan Res.* **2017**, *10*, DOI: 10.1177/1178646917691938.

66. Bertazzo, A.; Ragazzi, E.; Biasiolo, M.; Costa, C.V.; Allegri, G. Enzyme activities involved in tryptophan metabolism along the kynurenine pathway in rabbits. *Biochim. Biophys. Acta - Biomembr. -General Sub.* **2001**, *1527*, 167–175. DOI: 10.1016/S0304-4165(01)00165-9.

67. Miller, C.L.; Llenos, I.C.; Dulay, J.R.; Barillo, M.M.; Yolken, R.H.; Weis, S. Expression of the kynurenine pathway enzyme tryptophan 2, 3-dioxygenase is increased in the frontal cortex of individuals with schizophrenia. *Neurobiol. Dis.* **2004**, *15*, 618–629. DOI: 10.1016/j.nbd.2003.12.015.

68. Fedorova, M.; Todorovsky, T.; Kuleva, N.; Hoffmann, R. Quantitative evaluation of tryptophan oxidation in actin and troponin I from skeletal muscles using a rat model of acute oxidative stress. *Proteomics* **2010**, *10*, 2692–2700. DOI: 10.1002/pmic.201000147.

69. Rexroth, S.; Poetsch, A.; Rögner, M.; Hamann, A.; Werner, A.; Osiewacz, H.D.; Schäfer, E.R.; Seelert, H.; Dencher, N.A. Reactive oxygen species target specific tryptophan site in the mitochondrial ATP synthase. *Biochim Biophys Acta-Bioenerg.* **2012**, *1817*, 381–387. DOI: 10.1016/j.bbabio.2011.11.006.

70. Ehrenshaft, M.; Deterding, L.J.; Mason, R.P.; Medicine. Tripping up Trp: Modification of protein tryptophan residues by reactive oxygen species, modes of detection, and biological consequences. *Free Radic. Biol.* **2015**, *89*, 220–228. DOI: 10.1016/j.freeradbiomed.2015.08.003.

71. Crotti, S.; Enzo, M.V.; Bedin, C.; Pucciarelli, S.; Maretto, I.; Del Bianco, P.; Traldi, P.; Tasciotti, E.; Ferrari, M.; Rizzolio, F. Clinical predictive circulating peptides in rectal cancer patients treated with neoadjuvant chemoradiotherapy. *J. Cell. Physiol.* **2015**, *230*, 1822–1828. DOI: 10.1002/jcp.24894.

72. Lucki, I. The spectrum of behaviors influenced by serotonin. *Biol. Psychiatry* **1998**, *44*, 151–162. DOI: 10.1016/S0006-3223(98)00139-5.

73. Comai, S.; Gobbi, G. CCNP Award Paper: Unveiling the role of melatonin MT2 receptors in sleep, anxiety and other neuropsychiatric diseases: a novel target in psychopharmacology. *J. Psychiatry. Neurosci.* **2014**, *39*, 6–21. DOI: 10.1503/jpn.130009.

74. Jockers, R.; Delagrange, P.; Dubocovich, M.L.; Markus, R.P.; Renault, N.; Tosini, G.; Cecon, E.; Zlotos, D.P. Update on melatonin receptors: IUPHAR Review 20. *Br. J. Pharmacol.* **2016**, *173*, 2702–2725. DOI: 10.1111/bph.13536.

75. Rogers, S.R.; Pesti, G.M. The influence of dietary tryptophan on broiler chick growth and lipid metabolism as mediated by dietary protein levels. *Poult. Sci.* **1990**, *69*, 746–756. DOI: 10.3382/ps.0690746.

76. Fears, R.; Murrellt, E.A. Tryptophan and the control of triglyceride and carbohydrate metabolism in the rat. *Br. J. Nutr.* **1980**, *43*, 349–356. DOI: 10.1079/BJN19800097.

77. Ruan, Z.; Yang, Y.; Zhou, Y.; Wen, Y.; Ding, S.; Liu, G.; Wu, X.; Liao, P.; Deng, Z.; Assaad, H. Metabolomic analysis of amino acid and energy metabolism in rats supplemented with chlorogenic acid. *Amino Acids* **2014**, *46*, 2219–2229. DOI: 10.1007/s00726-014-1762-7.

78. Eleftheriadis, T.; Pissas, G.; Sounidaki, M.; Tsogka, K.; Antoniadis, N.; Antoniadi, G.; Liakopoulos, V.; Stefanidis, I. Indoleamine 2, 3-dioxygenase, by degrading L-tryptophan, enhances carnitine palmitoyltransferase I activity and fatty acid oxidation, and exerts fatty acid-dependent effects in human alloreactive CD4+ T-cells. *Int. J. Mol. Med.* **2016**, *38*, 1605–1613. DOI: 10.3892/ijmm.2016.2750.

79. Sun, H.; Huang, F.-f.; Qu, S. Melatonin: a potential intervention for hepatic steatosis. *Lipids Health Dis.* **2015**, *14*, 1–6. DOI: 10.1186/s12944-015-0081-7.

80. Hedo, J.; Villanueva, M.L.; Marco, J. Elevation of plasma glucose and glucagon after tryptophan ingestion in man. *Metabolism* **1977**, *26*, 1131–1134. DOI: 10.1016/0026-0495(77)90040-3.

81. Inubushi, T.; Kamemura, N.; Oda, M.; SAkURAI, J.; NAkAYA, Y.; Harada, N.; Suenaga, M.; Matsunaga, Y.; Ishidoh, K.; Katunuma, N. L-tryptophan suppresses rise in blood glucose and

preserves insulin secretion in type-2 diabetes mellitus rats. *J. Nutr. Sci. Vitaminol.* **2012**, *58,* 415–422. DOI: 10.3177/jnsv.58.415.

82. Sugimoto, Y.; Kimura, I.; Yamada, J.; Watanabe, Y.; Takeuchi, N.; Horisaka, K. The involvement of insulin in tryptamine-induced hypoglycemia in mice. *Life Sci.* **1991**, *48,* 1679–1683. DOI: 10.1016/0024-3205(91)90128-X.

83. Agil, A.; Rosado, I.; Ruiz, R.; Figueroa, A.; Zen, N.; Fernández-Vázquez, G. Melatonin improves glucose homeostasis in young Zucker diabetic fatty rats. *J. Pineal Res.* **2012**, *52,* 203–210. DOI: 10.1111/j.1600-079X.2011.00928.x.

84. Lima, F.B.; Machado, U.F.; Bartol, I.; Seraphim, P.M.; Sumida, D.H.; Moraes, S.M.; Hell, N.S.; Okamoto, M.M.; Saad, M.J.; Carvalho, C.R. Pinealectomy causes glucose intolerance and decreases adipose cell responsiveness to insulin in rats. *Am. J. Physiol. Endocrinol. Metab.*

1998, E934–E941. DOI: 10.1152/ajpendo.1998.275.6.E934.

85. Diaz, B.; Blazquez, E. Effect of pinealectomy on plasma glucose, insulin and glucagon levels in the rat. *Horm. Metab. Res.* **1986**, *18,* 225–229.

86. Nogueira, T.C.; Lellis-Santos, C.; Jesus, D.S.; Taneda, M.; Rodrigues, S.C.; Amaral, F.G.; Lopes, A.M.S.; Cipolla-Neto, J.; Bordin, S.; Anhê, G.F. Absence of melatonin induces night-time hepatic insulin resistance and increased gluconeogenesis due to stimulation of nocturnal unfolded protein response. *Endocrinology* **2011**, *152,* 1253–1263. DOI: 10.1210/en.2010-1088.

87. Tresguerres, J.A.; Cuesta, S.; Kireev, R.A.; Garcia, C.; Acuña-Castroviejo, D.; Vara, E. Beneficial effect of melatonin treatment on age-related insulin resistance and on the development of type 2 diabetes. *Horm. Mol. Biol. Clin. Investig.* **2013**, *16,* 47–54. DOI: 10.1515/hmbci-2013-0041.

88. Tudhope, S.J.; Wang, C.-C.; Petrie, J.L.; Potts, L.; Malcomson, F.; Kieswich, J.; Yaqoob, M.M.; Arden, C.; Hampson, L.J.; Agius, L. A novel mechanism for regulating hepatic glycogen synthesis involving serotonin and cyclin-dependent kinase-5. *Diabetes* **2012**, *61*, 49–60. DOI: 10.2337/db11-0870.

89. Moore, M.C.; Kimura, K.; Shibata, H.; Honjoh, T.; Saito, M.; Everett, C.A.; Smith, M.S.; Cherrington, A.D. Portal 5-hydroxytryptophan infusion enhances glucose disposal in conscious dogs. *Am. J. Physiol. Endocrinol. Metab.* **2005**, *289*, E225–E231. DOI: 10.1152/ajpendo.00614.2004.

90. Hajduch, E.; Rencurel, F.; Balendran, A.; Batty, I.H.; Downes, C.P.; Hundal, H.S. Serotonin (5-hydroxytryptamine), a novel regulator of glucose transport in rat skeletal muscle. *J. Biol. Chem.* **1999**, *274*, 13563–13568. DOI: 10.1074/jbc.274.19.13563.

91. McDaniel, E.; Hundley, J.; Sebrell, W. Tryptophan-Niacin Metabolism in Alloxan Diabetic Rats: Two Figures. *J. Nutr.* **1956**, *59*, 407–423. DOI: 10.1093/jn/59.3.407.

92. Ray, P.D.; Foster, D.O.; Lardy, H.A. Paths of carbon in gluconeogenesis and lipogenesis: IV. Inhibition by L-tryptophan of hepatic gluconeogenesis at the level of phosphoenolpyruvate formation. *J. Biol. Chem.* **1966**, *241*, 3904–3908. DOI: 10.1016/S0021-9258(18)99792-9.

93. Foster, D.O.; Ray, P.D.; Lardy, H.A. A paradoxical in vivo effect of L-tryptophan on the phosphoenolpyruvate carboxykinase of rat liver. *Biochemistry* **1966**, *5*, 563–569.

94. Rippe, D.F.; Berry, L.J. Effect of endotoxin on the activation of phosphoenolpyruvate carboxykinase by tryptophan. *Infection Immunity* **1972**, *6*, 97–98.

95. Ardiansyah, S.; Shirakawa, H.; Inagawa, Y.; Koseki, T.; Komai, M. Regulation of blood pressure and glucose metabolism induced by L-tryptophan in stroke-prone spontaneously hypertensive rats. *Nutr. Metab.* **2011**, *8*, 1–7. DOI: 10.1186/1743-7075-8-45.

96. Ji, K.; Liang, H.; Chisomo-Kasiya, H.; Mokrani, A.; Ge, X.; Ren, M.; Liu, B. Effects of dietary tryptophan levels on growth performance, whole body composition and gene expression levels related to glycometabolism for juvenile blunt snout bream, Megalobrama amblycephala. *Aquacult. Nutr.* **2018**, *24,* 1474–1483. DOI: 10.1111/anu.12684.

97. Rubin, B.Y.; Anderson, S.L.; Xing, L.; Powell, R.J.; Tate, W. Interferon induces tryptophanyl-tRNA synthetase expression in human fibroblasts. *J. Biol. Chem.* **1991**, *266,* 24245–24248. DOI: 10.1016/S0021-9258(18)54219-8.

98. Jin, M. Unique roles of tryptophanyl-tRNA synthetase in immune control and its therapeutic implications. *Exp. Mol. Med.* **2019**, *51,* 1–10. DOI: 10.1038/s12276-018-0196-9.

99. Nørgaard, J.; Pedersen, T.; Soumeh, E.; Blaabjerg, K.; Canibe, N.; Jensen, B.; Poulsen, H. Optimum standardized ileal digestible tryptophan to lysine ratio for pigs weighing 7–14 kg. *Livest. Sci.* **2015**, *175,* 90–95. DOI: 10.1016/j.livsci.2015.02.012.

100. De Ridder, K.; Levesque, C.; Htoo, J.; De Lange, C. Immune system stimulation reduces the efficiency of tryptophan utilization for body protein deposition in growing pigs. *J. Anim. Sci.* **2012**, *90,* 3485–3491. DOI: 10.2527/jas.2011-4830.

101. Fürst, P.; Guarnieri, G.; Hultman, E. The effect of the administration of L-tryptophan on synthesis of urea and gluconeogenesis in man. *Scand. J. Clin. Lab. Invest.* **1971**, *27,* 183–191. DOI: 10.3109/00365517109080206.

102. Pátek, M. Branched-chain amino acids. In *Amino Acid Biosynthesis~ Pathways, Regulation and Metabolic Engineering*, 2007, 129–162.

103. Adeva, M.M.; Calviño, J.; Souto, G.; Donapetry, C. Insulin resistance and the metabolism of branched-chain amino acids in humans. *Amino Acids* **2012**, *43,* 171–181. DOI: 10.1007/s00726-011-1088-7.

104. Barnes, M.R.; Gray, I.C., *Bioinformatics for geneticists*. 2003, England: John Wiley and Sons.

105. Brosnan, J.T.; Brosnan, M.E. Branched-chain amino acids: enzyme and substrate regulation. *J. Nutr.* **2006**, *136,* 207S–211S. DOI: 10.1093/jn/136.1.207S.

106. Gu, C.; Mao, X.; Chen, D.; Yu, B.; Yang, Q. Isoleucine plays an important role for maintaining immune function. *Curr Protein Pept Sci* **2019**, *20,* 644–651. DOI: 10.2174/1389203720666190305163135.

107. Kainulainen, H.; Hulmi, J.J.; Kujala, U.M.J.E.; reviews, s.s. Potential role of branched-chain amino acid catabolism in regulating fat oxidation. **2013**, *41,* 194-200.

108. Zhang, S.; Zeng, X.; Ren, M.; Mao, X.; Qiao, S. Novel metabolic and physiological functions of branched chain amino acids: a review. *J. Anim. Sci. Biotechnol.* **2017**, *8,* 1–12. DOI: 10.1186/s40104-016-0139-z.

109. Sperringer, J.E.; Addington, A.; Hutson, S.M. Branched-chain amino acids and brain metabolism. *Neurochem. Res.* **2017**, *42,* 1697–1709. DOI: 10.1007/s11064-017-2261-5.

110. Kadowaki, T.; Yamauchi, T. Adiponectin and adiponectin receptors. *Endocr. Rev.* **2005**, *26,* 439–451. DOI: 10.1210/er.2005-0005.

111. Nishimura, J.; Masaki, T.; Arakawa, M.; Seike, M.; Yoshimatsu, H. Isoleucine prevents the accumulation of tissue triglycerides and upregulates the expression of PPARα and uncoupling protein in diet-induced obese mice. *J. Nutr.* **2010**, *140,* 496–500. DOI: 10.3945/jn.109.108977.

112. Feldmann, H.M.; Golozoubova, V.; Cannon, B.; Nedergaard, J. UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metab.* **2009**, *9,* 203–209. DOI: 10.1016/j.cmet.2008.12.014.

113. Masaki, T.; Yoshimatsu, H.; Chiba, S.; Sakata, T. Impaired response of UCP family to cold exposure in diabetic (db/db) mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2000**, *279*, R1305–R1309. DOI: 10.1152/ajpregu.2000.279.4.R1305.

114. Zhao, Y.; Dong, X.; Guo, C.-R.; Feng, L.; Liu, Y.; Jiang, W.-D.; Wu, P.; Luo, W.; Huang, X.-L.; Chen, D.-F. Dietary isoleucine affects muscle fatty acid and amino acid profiles through regulating lipid metabolism and autophagy in hybrid catfish Pelteobagrus vachelli♀× Leiocassis longirostris♂. *Anim. Nutr.* **2022**, DOI: 10.1016/j.aninu.2022.07.006.

115. Green, C.R.; Wallace, M.; Divakaruni, A.S.; Phillips, S.A.; Murphy, A.N.; Ciaraldi, T.P.; Metallo, C.M. Branched-chain amino acid catabolism fuels adipocyte differentiation and lipogenesis. *Nat. Chem. Biol.* **2016**, *12*, 15–21. DOI: 10.1038/nchembio.1961.

116. Luo, Y.; Zhang, X.; Zhu, Z.; Jiao, N.; Qiu, K.; Yin, J. Surplus dietary isoleucine intake enhanced monounsaturated fatty acid synthesis and fat accumulation in skeletal muscle of finishing pigs. *J. Anim. Sci. Biotechnol.* **2018**, *9*, 1–11. DOI: 10.1186/s40104-018-0306-5.

117. Doi, M.; Yamaoka, I.; Fukunaga, T.; Nakayama, M. Isoleucine, a potent plasma glucose-lowering amino acid, stimulates glucose uptake in C2C12 myotubes. *Biochem. Biophys. Res. Commun.* **2003**, *312*, 1111–1117. DOI: 10.1016/j.bbrc.2003.11.039.

118. Doi, M.; Yamaoka, I.; Nakayama, M.; Sugahara, K.; Yoshizawa, F. Hypoglycemic effect of isoleucine involves increased muscle glucose uptake and whole body glucose oxidation and decreased hepatic gluconeogenesis. *Am. J. Physiol. - Endocrinol. Metab.* **2007**, *292*, E1683–E1693. DOI: 10.1152/ajpendo.00609.2006.

119. Nishitani, S.; Takehana, K.; Fujitani, S.; Sonaka, I. Branched-chain amino acids improve glucose metabolism in rats with liver cirrhosis. *Am. J. Physiol. - Gastrointest. Liver Physiol.* **2005**, *288*, G1292–G1300. DOI: 10.1152/ajpgi.00510.2003.

120. Parr, T.; Kerr, B.; Baker, D. Isoleucine requirement of growing (25 to 45 kg) pigs. *J. Anim. Sci.* **2003**, *81*, 745–752. DOI: 10.2527/2003.813745x.

121. Wiltafsky, M.; Bartelt, J.; Relandeau, C.; Roth, F. Estimation of the optimum ratio of standardized ileal digestible isoleucine to lysine for eight-to twenty-five-kilogram pigs in diets containing spray-dried blood cells or corn gluten feed as a protein source. *J. Anim. Sci.* **2009**, *87*, 2554–2564. DOI: 10.2527/jas.2008-1320.

122. Oestemer, G.; Hanson, L.; Meade, R. Reevaluation of the isoleucine requirement of the young pig. *J. Anim. Sci.* **1973**, *36*, 679–683. DOI: 10.2527/jas1973.364679x.

123. Coude, F.; Sweetman, L.; Nyhan, W. Inhibition by propionyl-coenzyme A of N-acetylglutamate synthetase in rat liver mitochondria. A possible explanation for hyperammonemia in propionic and methylmalonic acidemia. *J. Clin. Investig.* **1979**, *64*, 1544–1551. DOI: 10.1172/JCI109614.

124. Stewart, P.M.; Walser, M. Failure of the normal ureagenic response to amino acids in organic acid-loaded rats: Proposed mechanism for the hyperammonemia of propionic and methylmalonic acidemia. *J. Clin. Investig.* **1980**, *66*, 484–492. DOI: 10.1172/JCI109879.

125. Scholl-Bürgi, S.; Sass, J.O.; Zschocke, J.; Karall, D. Amino acid metabolism in patients with propionic acidaemia. *J. Inherited Metab. Dis.* **2012**, *35*, 65–70. DOI: 10.1007/s10545-010-9245-9.

126. Nørgaard, J.; Fernández, J. Isoleucine and valine supplementation of crude protein-reduced diets for pigs aged 5–8 weeks. *Anim. Feed Sci. Technol.* **2009**, *154*, 248–253. DOI: 10.1016/j.anifeedsci.2009.08.010.

127. Zhang, X.; Liu, X.; Jia, H.; He, P.; Mao, X.; Qiao, S.; Zeng, X. Valine supplementation in a reduced protein diet regulates growth performance partially through modulation of plasma amino

acids profile, metabolic responses, endocrine, and neural factors in piglets. *J. Agric. Food Chem.* **2018**, *66*, 3161–3168. DOI: 10.1021/acs.jafc.8b01113.

128. Ma, W.; Mao, P.; Fan, W.; Zhu, Y.; Guo, L. Valine and isoleucine supplementation improve performance and serum biochemical concentrations in growing gilts fed low-protein diets. *Can J Anim Sci* **2019**, *99*, 921-928.

129. Roux, M.; Donsbough, A.; Waguespack, A.; Powell, S.; Bidner, T.; Payne, R.; Southern, L. Maximizing the use of supplemental amino acids in corn-soybean meal diets for 20-to 45-kilogram pigs. *J. Anim. Sci.* **2011**, *89*, 2415–2424. DOI: 10.2527/jas.2010-3756.

130. Escobar, J.; Frank, J.W.; Suryawan, A.; Nguyen, H.V.; Kimball, S.R.; Jefferson, L.S.; Davis, T.A. Regulation of cardiac and skeletal muscle protein synthesis by individual branched-chain amino acids in neonatal pigs. *Am. J. Physiol. - Endocrinol. Metab.* **2006**, *290*, E612–E621. DOI: 10.1152/ajpendo.00402.2005.

131. Tischler, M.E.; Desautels, M.; Goldberg, A.L. Does leucine, leucyl-tRNA, or some metabolite of leucine regulate protein synthesis and degradation in skeletal and cardiac muscle? *J. Biol. Chem.* **1982**, *257*, 1613–1621. DOI: 10.1016/S0021-9258(19)68081-6

132. Chen, X.-h.; Liu, S.-r.; Peng, B.; Li, D.; Cheng, Z.-x.; Zhu, J.-x.; Zhang, S.; Peng, Y.-m.; Li, H.; Zhang, T.-t. Exogenous L-valine promotes phagocytosis to kill multidrug-resistant bacterial pathogens. *Front. Immunol.* **2017**, *8*, 207. DOI: 10.3389/fimmu.2017.00207.

133. Che, L.; Xu, M.; Gao, K.; Zhu, C.; Wang, L.; Yang, X.; Wen, X.; Xiao, H.; Jiang, Z.; Wu, D.J.B.O.R. Valine increases milk fat synthesis in mammary gland of gilts through stimulating AKT/MTOR/SREBP1 pathway. *Biol. Reprod.* **2019**, *101*, 126–137. DOI: 10.1093/biolre/ioz065.

134. Claessens, M.; Saris, W.H.; Bouwman, F.G.; Evelo, C.T.; Hul, G.B.; Blaak, E.E.; Mariman, E.C. Differential valine metabolism in adipose tissue of low and high fat-oxidizing obese subjects. *Proteomics Clin. Appl.* **2007**, *1*, 1306–1315. DOI: 10.1002/prca.200700049.

135. Du, Y.; Meng, Q.; Zhang, Q.; Guo, F. Isoleucine or valine deprivation stimulates fat loss via increasing energy expenditure and regulating lipid metabolism in WAT. *Amino Acids* **2012**, *43*, 725–734. DOI: 10.1007/s00726-011-1123-8.

136. Asghari, G.; Farhadnejad, H.; Teymoori, F.; Mirmiran, P.; Tohidi, M.; Azizi, F. High dietary intake of branched-chain amino acids is associated with an increased risk of insulin resistance in adults. *J. Diabetes* **2018**, *10*, 357–364. DOI: 10.1111/1753-0407.12639.

137. Craig, A.; Henry, W.; Magowan, E. Effect of phase feeding and valine-to-lysine ratio during lactation on sow and piglet performance. *J. Anim. Sci.* **2016**, *94*, 3835–3843. DOI: 10.2527/jas.2016-0648

138. Ma, Q.; Hu, L.; Zhu, J.; Chen, J.; Wang, Z.; Yue, Z.; Qiu, M.; Shan, A. Valine supplementation does not reduce lipid accumulation and improve insulin sensitivity in mice fed high-fat diet. *ACS omega* **2020**, *5*, 30937–30945. DOI: 10.1021/acsomega.0c03707.

139. Fukuchi, S.; Hamaguchi, K.; Seike, M.; Himeno, K.; Sakata, T.; Yoshimatsu, H. Role of fatty acid composition in the development of metabolic disorders in sucrose-induced obese rats. *Exp. Biol. Med.* **2004**, *229*, 486–493. DOI: 10.1177/153537020422900

140. Buettner, R.; Parhofer, K.; Woenckhaus, M.; Wrede, C.; Kunz-Schughart, L.A.; Scholmerich, J.; Bollheimer, L.J.J.o.m.e. Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. *J. Mol. Endocrinol.* **2006**, *36*, 485-501. DOI: 10.1677/jme.1.01909

141. Wan, J.; Jiang, L.; Lü, Q.; Ke, L.; Li, X.; Tong, N. Activation of PPARδ up-regulates fatty acid oxidation and energy uncoupling genes of mitochondria and reduces palmitate-induced apoptosis in pancreatic β-cells. *Biochem. Biophys. Res. Commun.* **2010**, *391*, 1567–1572. DOI: 10.1016/j.bbrc.2009.12.127.

142. Coskun, T.; Bina, H.A.; Schneider, M.A.; Dunbar, J.D.; Hu, C.C.; Chen, Y.; Moller, D.E.; Kharitonenkov, A. Fibroblast growth factor 21 corrects obesity in mice. *Endocrinology* **2008**, *149*, 6018–6027. DOI: 10.1210/en.2008-0816.

143. Lin, Z.; Tian, H.; Lam, K.S.; Lin, S.; Hoo, R.C.; Konishi, M.; Itoh, N.; Wang, Y.; Bornstein, S.R.; Xu, A. Adiponectin mediates the metabolic effects of FGF21 on glucose homeostasis and insulin sensitivity in mice. *Cell Metab.* **2013**, *17*, 779–789. DOI: 10.1016/j.cmet.2013.04.005.

144. Badman, M.K.; Pissios, P.; Kennedy, A.R.; Koukos, G.; Flier, J.S.; Maratos-Flier, E. Hepatic fibroblast growth factor 21 is regulated by PPARα and is a key mediator of hepatic lipid metabolism in ketotic states. *Cell Metab.* **2007**, *5*, 426–437. DOI: 10.1016/j.cmet.2007.05.002.

145. Inagaki, T.; Dutchak, P.; Zhao, G.; Ding, X.; Gautron, L.; Parameswara, V.; Li, Y.; Goetz, R.; Mohammadi, M.; Esser, V. Endocrine regulation of the fasting response by PPARα-mediated induction of fibroblast growth factor 21. *Cell Metab.* **2007**, *5*, 415–425. DOI: 10.1016/j.cmet.2007.05.003.

146. Bishop, C.A.; Schulze, M.B.; Klaus, S.; Weitkunat, K. The branched-chain amino acids valine and leucine have differential effects on hepatic lipid metabolism. *The FASEB Journal* **2020**, *34*, 9727–9739. DOI: 10.1096/fj.202000195R.

147. Imamura, F.; Fretts, A.; Marklund, M.; Ardisson Korat, A.V.; Yang, W.-S.; Lankinen, M.; Qureshi, W.; Helmer, C.; Chen, T.-A.; Wong, K. Fatty acid biomarkers of dairy fat consumption

and incidence of type 2 diabetes: a pooled analysis of prospective cohort studies. *PLoS Med.* **2018**, *15*, e1002670. DOI: 10.1371/journal.pmed.1002670.

148. Jang, C.; Oh, S.F.; Wada, S.; Rowe, G.C.; Liu, L.; Chan, M.C.; Rhee, J.; Hoshino, A.; Kim, B.; Ibrahim, A. A branched-chain amino acid metabolite drives vascular fatty acid transport and causes insulin resistance. *Nat. Med.* **2016**, *22*, 421–426. DOI: 10.1038/nm.4057.

149. Arrieta-Cruz, I.; Su, Y.; Gutiérrez-Juárez, R. Suppression of endogenous glucose production by isoleucine and valine and impact of diet composition. *Nutrients* **2016**, *8*, 79. DOI: 10.3390/nu8020079.

150. Liao, X.; Liu, B.; Qu, H.; Zhang, L.; Lu, Y.; Xu, Y.; Lyu, Z.; Zheng, H. A high level of circulating valine is a biomarker for type 2 diabetes and associated with the hypoglycemic effect of sitagliptin. *Mediators Inflamm.* **2019**, *2019*, DOI: 10.1155/2019/8247019.

151. Elovaris, R.A.; Bitarafan, V.; Agah, S.; Ullrich, S.S.; Lange, K.; Horowitz, M.; Feinle-Bisset, C. Comparative Effects of the Branched-Chain Amino Acids, Leucine, Isoleucine and Valine, on Gastric Emptying, Plasma Glucose, C-Peptide and Glucagon in Healthy Men. *Nutrients* **2021**, *13*, 1613. DOI: 10.3390/nu13051613.

152. Kimball, S.R.; Jefferson, L.S. Regulation of protein synthesis by branched-chain amino acids. *Curr. Opin. Clin. Nutr. Metab. Care* **2001**, *4*, 39–43.

153. Saheki, T.; Sato, Y.; Takada, S. Regulation of Urea Synthesis in Rat Liver Inhibition of Urea Synthesis by L-Norvaline. *J. Biochem.* **1979**, *86*, 745–750.

154. Bode, A.M.; Foster, J.D.; Nordlie, R.C. Glycogenesis from glucose and ureagenesis in isolated perfused rat livers. Influence of ammonium ion, norvaline, and ethoxyzolamide. *J. Biol. Chem.* **1994**, *269*, 7879–7886. DOI: 10.1016/S0021-9258(17)37133-8.

155. Polis, B.; Samson, A.O. A new perspective on Alzheimer's disease as a brain expression of a complex metabolic disorder. *Alzheimer's Disease [Internet]* **2019**, DOI: 10.15586/alzheimersdisease.2019.ch1.

156. Katunuma, N.; Okada, M.; Nishii, Y. Regulation of the urea cycle and TCA cycle by ammonia. *Adv. Enzyme Regul.* **1966**, *4*, 317–335. DOI: 10.1016/0065-2571(66)90025-2.

157. Liu, Y.; Wang, X.; Hou, Y.; Yin, Y.; Qiu, Y.; Wu, G.; Hu, C.-A.A. Roles of amino acids in preventing and treating intestinal diseases: recent studies with pig models. *Amino Acids* **2017**, *49*, 1277–1291. DOI: 10.1007/s00726-017-2450-1.

158. Verma, N.; Rettenmeier, A.W.; Schmitz-Spanke, S. Recent advances in the use of Sus scrofa (pig) as a model system for proteomic studies. *Proteomics* **2011**, *11*, 776–793. DOI: 10.1002/pmic.201000320.

159. Clouard, C.; Meunier-Salaün, M.-C.; Val-Laillet, D. Food preferences and aversions in human health and nutrition: how can pigs help the biomedical research? *Animal* **2012**, *6*, 118–136. DOI: 10.1017/S1751731111001315.

160. Spurlock, M.E.; Gabler, N.K. The development of porcine models of obesity and the metabolic syndrome. *J. Nutr.* **2008**, *138*, 397–402. DOI: 10.1093/jn/138.2.397.

161. Eberhard, M.; Hennig, U.; Kuhla, S.; Brunner, R.M.; Kleessen, B.; Metges, C.C. Effect of inulin supplementation on selected gastric, duodenal, and caecal microbiota and short chain fatty acid pattern in growing piglets. *Arch. Anim. Nutr.* **2007**, *61*, 235–246. DOI: 10.1080/17450390701431631.

162. Hou, N.; Du, X.; Wu, S. Advances in pig models of human diseases. *Exp. Anim.* **2022**, *5*, 141–152. DOI: 10.1002/ame2.12223.

163. Renner, S.; Braun-Reichhart, C.; Blutke, A.; Herbach, N.; Emrich, D.; Streckel, E.; Wünsch, A.; Kessler, B.; Kurome, M.; Bähr, A. Permanent neonatal diabetes in INS C94Y transgenic pigs. *Diabetes* **2013**, *62*, 1505–1511. DOI: 10.2337/db12-1065.

164. Cho, B.; Kim, S.J.; Lee, E.-J.; Ahn, S.M.; Lee, J.S.; Ji, D.-Y.; Lee, K.; Kang, J.-T. Generation of insulin-deficient piglets by disrupting INS gene using CRISPR/Cas9 system. *Transgenic Res.* **2018**, *27*, 289–300. DOI: 10.1007/s11248-018-0074-1.

165. Benevenga, N. Amino acid metabolism in swine: applicability to normal and altered amino acid metabolism in humans. **1986**, 1017–1030.

166. Oli, M.; Petschow, B.; Buddington, R. Evaluation of fructooligosaccharide supplementation of oral electrolyte solutions for treatment of diarrhea (Recovery of the intestinal bacteria). *Dig. Dis. Sci.* **1998**, *43*, 138–147. DOI: 10.1023/A:1018892524790.

167. Andersen, A.; Mølbak, L.; Thymann, T.; Michaelsen, K.; Lauritzen, L. Dietary long-chain n-3 PUFA, gut microbiota and fat mass in early postnatal piglet development—exploring a potential interplay. *Prostaglandins Leukot. Essent. Fat. Acids* **2011**, *85*, 345–351. DOI: 10.1016/j.plefa.2011.08.004.

CHAPTER III

DIETARY TRYPTOPHAN SUPPLEMENTATION ALTERS FAT AND GLUCOSE METABOLISM IN A LOW-BIRTHWEIGHT PIGLET MODEL

This Chapter is based on: *Parniyan Goodarzi, Mohammad Habibi, Kennedy Roberts, Julia Sutton, Cedrick Ndhumba Shili, Dingbo Lin and Adel Pezeshki. Dietary Tryptophan Supplementation Alters Fat and Glucose Metabolism in a Low-Birthweight Piglet Model. Nutrients, 13, 2561*. 2561. https://doi.org/ 10.3390/nu13082561

3.1. Abstract

Low birthweight (LBW) is associated with metabolic complications, such as glucose and lipid metabolism disturbances in early life. The objective of this study was to assess: (1) the effect of dietary tryptophan (Trp) on glucose and fat metabolism in an LBW piglet model, and (2) the role peripheral 5-hydroxytryptamine type 3 (5HT3) receptors in regulating the feeding behavior in LBW piglets fed with Trp-supplemented diets. Seven-day-old piglets were assigned to 4 treatments: normal birthweight-0%Trp (NBW-T0), LBW-0%Trp (LBW-T0), LBW-0.4%Trp (LBW-T0.4), and LBW-0.8%Trp (LBW-T0.8) for 3 weeks. Compared to LBW-T0, the blood glucose was decreased in LBW-T0.8 at 60 min following the meal test, and the triglycerides were lower in LBW-T0.4 and LBW-T0.8. Relative to LBW-T0, LBW-T0.8 had a lower transcript and protein abundance of hepatic glucose transporter-2, a higher mRNA abundance of glucokinase, and a lower transcript of phosphoenolpyruvate carboxykinase.

LBW-T0.4 tended to have a lower protein abundance of sodium-glucose co-transporter 1 in the jejunum. In comparison with LBW-T0, LBW-T0.4 and LBW-T0.8 had a lower transcript of hepatic acetyl-CoA carboxylase, and LBW-T0.4 had a higher transcript of 3-hydroxyacyl-CoA dehydrogenase. Blocking 5-HT3 receptors with ondansetron reduced the feed intake in all groups, with a transient effect on LBW-T0, but more persistent effect on LBW-T0.8 and NBW-T0. In conclusion, Trp supplementation reduced the hepatic lipogenesis and gluconeogenesis, but increased the glycolysis in LBW piglets. Peripheral serotonin is likely involved in the regulation of feeding behavior, particularly in LBW piglets fed diets supplemented with a higher dose of Trp.

3.2. Introduction

Newborns who fail to reach an estimated fetal biometric by a certain gestational age (i.e., below the 10th percentile for gestational age) are considered small for gestational age [1]. The incidence of low-birthweight (LBW) infants, weighing less than 2500 g at birth, is 8.31% in the United States, with one third of these infants being recognized as intrauterine growth retarded (IUGR) [2–4]. The LBW is not only associated with a higher risk of mortality and morbidity and the development of complications, such as insulin resistance, cardiovascular disease, hypertension, adverse lipid metabolism, dyslipidemia, and obesity in the long term [5–8], but also related with metabolic disturbances of glucose and lipid metabolism in the short term [9,10]. IUGR rats show alterations in the expression of genes involved in hepatic energy production [11] and exhibit hyperglycemia due to an increased gluconeogenesis as a result of impaired mitochondrial oxidative phosphorylation and pyruvate oxidation in the liver [12]. Similarly, LBW infants [13] and IUGR pigs [14,15] develop hyperglycemia in early life. In addition to impairment in glucose metabolism, the utilization of circulating triglycerides is compromised in LBW infants [16]. Further, a Nutrients 2021, 13, 2561. https://doi.org/10.3390/nu13082561 https://www.mdpi.com/journal/nutrients Nutrients 2021, 13, 2561 2 of 22 lower insulin sensitivity and less optimized lipid metabolism in early postnatal life have been reported in LBW infants [9]. Similarly, a low activity for hepatic

lipoprotein lipase has been shown in IUGR piglets in comparison to their normal-weight counterparts [17]. Therefore, there is evidence that glucose and lipid metabolism is impaired in LBW neonates during early life, which may ultimately lead to the occurrence of metabolic disorders in the short term and long term. Hence, efficient strategies need to be developed to improve the glucose and lipid metabolism in LBW infants during the early postnatal period. Nutrition is one of the important factors that can influence the glucose and lipid metabolism during the fetal and postnatal periods [8]. In particular, early postnatal nutrition is crucial for the optimal growth [8] and health of LBW infants. Further research is warranted to identify the role of supplemental amino acids (AA), which can improve the growth and glucose and lipid metabolism in LBW infants. Due to limitations regarding the ethical use of LBW infants for nutritional research, animal models are used to study the effect of nutritional interventions on metabolism in LBW offspring. LBW neonatal pigs have significant similarities with LBW infants in the structure and function of the gastrointestinal tract and nutrient metabolism and hence are used as models for LBW infants [18–21]. Tryptophan (Trp) is a neutral essential amino acid, which, due to its role in the regulation of various physiological functions, may potentially be used for developing therapeutics for preventing and treating chronic diseases [22]. Dietary Trp supplementation has been shown to reduce the serum lipids in rats [23] and the hepatic lipids in laying hens and broilers [24,25]. Further, dietary Trp suppress the hyperglycemia in rodents [22,26]. To our knowledge, no study has assessed the glucose and lipid metabolism of LBW neonates fed with milk-based diets supplemented with different doses of Trp. Trp, as a precursor of central serotonin, has been shown to have anorexigenic effects [27–30]. However, little is known on the role of peripheral serotonin in the regulation of feeding behavior. The effects of serotonin on appetite regulation are mediated by multiple receptor subtypes, including 5-hydroxytryptamine type 3 (5HT3) receptors [28,31]. It is unknown whether peripheral 5HT3 receptors are essential for feed intake regulation in LBW pigs fed with Trp-enriched diets. Therefore, the objective of this study was to investigate: (1) the effect of two different doses of dietary Trp on blood glucose and triglyceride concentrations and the mRNA and

protein expression of key markers related to lipid and glucose metabolism in an LBW piglet model, and (2) the role peripheral 5HT3 signaling in regulating the feeding behavior in LBW piglets fed with Trp-supplemented diets. Further, the effect of supplemental Trp on feed intake, growth measurements, thermal radiation, plasma metabolites, gut development, and hormones was assessed.

3.3. Materials and methods

3.3.1. Animals and housing

The experimental procedures used in this study were in accordance with the FASS Guide for the Care and Use of Agricultural Animals in Research and Teaching [32] and were approved by Oklahoma State University's Institutional Animal Care and Use Committee (IACUC; Animal Care and Use Protocol-IACUC-19-71). Seven-day-old male piglets were selected from twelve sows (Duroc sire line and Large White X Landrace dam; Seaboard, Hennessey, OK, USA) with a similar range of parity (2–4) and litter size (14–18). All piglets received the intramuscular injection of iron dextran complex (200 mg/mL) on day 3 postpartum (1 mL; 100 mg/kg body weight). The piglets were individually housed in plastic floor pens (0.86 × 0.79 × 0.81 m), equipped with milk replacer drinkers and a heat mat. The lighting program was based on a 16:8 h light: dark cycle [33], with lights on at 0900 and off at 0100 during the first week and then lights on at 0800 and off at 0000 in weeks 2 and 3 of the study, respectively. The room temperature was set at 30, 29, and 28 ◦C during week 1, 2, and 3 of the study, respectively.

3.3.2. Experimental Design and Diets

The body weight (BW) at day 7 postpartum was used as a criterion for considering pigs as LBW or normal birthweight (NBW). Seven-day-old piglets with a BW < 2 kg were considered as LBW, and piglets with a BW ≥ 2 kg were considered as NBW, in accordance with previous studies [34,35]. NBW was included in the experimental design as a control for the LBW animal model

used, regardless of the levels of Trp supplemented. The average BW of LBW and NBW piglets at arrival (i.e., day 7 after birth) were 1.66 ± 0.24 and 2.75 ± 0.47 kg, respectively. Prior to starting the experimental diets, following 3 days of adaptation, one NBW and three LBW piglets from each litter were assigned to one of following treatments, while maintaining the mean BW consistent for LBW pigs (2.93 ± 0.49 kg): (1) NBW-T0 (4.39 ± 0.45 kg, n = 8), NBW piglets fed a basal diet without supplemented Trp; (2) LBW-T0 (n = 8), LBW piglets fed a basal diet without supplemented Trp; (3) LBW-T0.4 (n = 7), LBW piglets fed a basal diet supplemented with 0.4% Trp; and (4) LBW-T0.8 (n = 8), LBW piglets fed a basal diet supplemented with 0.8% Trp for 3 weeks (Supplemental Table 3A.1). The experimental timeline is illustrated in Figure 3.1.

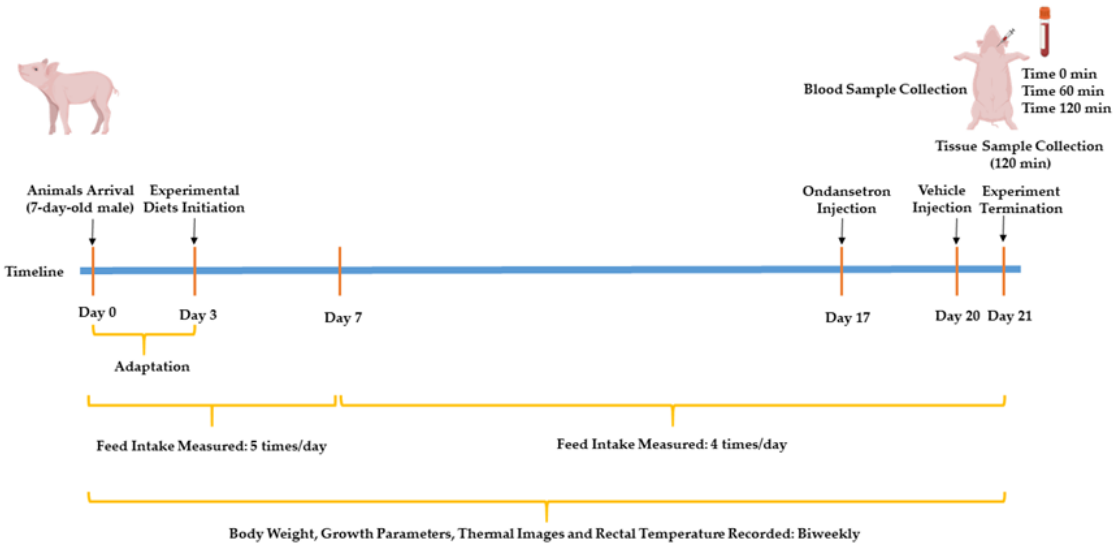


Figure 3.1. The experimental timeline.

Seven-day-old male piglets arrived on day zero and were assigned to low birthweight (LBW) or normal birthweight (NBW) groups, according to their body weight. Following three days of adaptation, LBW pigs were weight-matched, and then NBW and LBW pigs were subjected to one of four dietary treatments, including: NBW-T0, normal-birthweight piglets fed a basal diet without supplemented L-tryptophan (Trp); LBW-T0, low-birthweight piglets fed a basal diet without supplemented Trp; LBW-T0.4, low-birthweight piglets fed a basal diet supplemented with 0.4%

Trp; and LBW-T0.8, low-birthweight piglets fed a basal diet supplemented with 0.8% Trp. Following overnight fasting, all pigs received injections of ondansetron and saline on day 17 and 20, respectively. The feed intake was measured 5 times/day during the first week and 4 times/day during the last two weeks of the study. The body weight, growth parameters, thermal images, and rectal temperature were recorded biweekly throughout the study. After an overnight fast at week three, the pigs were allowed to consume their respective diets for 1 h (meal test). Blood samples were then collected at baseline and then at 60 and 120 min after the meal test. Following blood collection at 120 min after the meal test, all pigs were euthanized, and tissue samples were collected.

Milk replacer powder was formulated for piglets (Supplemental Table 3A.1), according to the nutrient requirements suggested by previous publications for neonatal pigs [36,37]. For the diet formulations, the chemical composition of the analyzed ingredients in this study (i.e., Arg, His, Phe, whey powder, and whey protein concentrate; Supplemental Tables 3A.2 and 3A.3) and our previous study was used [38]. Except for L-Trp, the amount of ingredients used across all three diets were kept consistent, and all diets were prepared as isonitrogenous and isocaloric by adding L-alanine and dextrose, respectively. A liquid diet was prepared by mixing 1 kg of milk replacer powder (dry matter: 95.6%) with 4.15 L of warm water (40 °C) to obtain a milk replacer with a similar dry matter to sow's milk, with 18.6% dry matter [39]. Fresh liquid milk replacers were prepared daily and stored at 4 °C throughout the day. Prior to feeding, the milk replacers were transferred to plastic bottles, warmed in a water bath (40 °C), and then offered to pigs in milk replacer feeders. Liquid milk was offered 5 times/day at 0900, 1400, 1900, 0000, and 0500 during the first week and then 4 times per day at 0800, 1400, 2000, and 0200 during weeks 2 and 3 of the study (Figure 3.1). The piglets had ad-libitum access to their diets during each meal and were provided a minimum 60 g dry matter/kg BW/day of milk replacer, as previously suggested [39,40].

3.3.3. Feed Intake and Growth Measurements

The milk replacer intake for each offering was measured by recording the volume of added milk to feeders and the leftover milk in the feeders, prior to the next feeding. Body weight and growth measurements, including heart girth, body length, and wither size, were recorded biweekly. The average daily gain (ADG; gained weight divided by experimental days), average dry matter intake (ADMI; consumed dry matter during feeding period divided by experimental days), average daily protein intake (ADPI; analyzed CP% × dry matter intake (DMI)/experimental days), gain:feed ratio (G:F; ADG divided by ADMI), and gain:protein ratio (G:P; ADG divided by ADPI) were calculated.

3.3.4. Thermal Images and Rectal Temperature

Individual thermal images were captured biweekly using a FLIR C2 compact thermal camera, with focal length of 1.54 mm and thermal accuracy of ±2 °C (FLIR Systems, Boston, MA, USA). The camera was positioned roughly 1 m above of each pig. The emissivity coefficient was set at 0.96. To measure the piglets' core body temperature, the rectal temperatures (RT) were measured biweekly for all piglets using an electronic thermometer.

3.3.5. Blockade of 5-hydroxytryptamine Type 3 (5HT3) Receptors

The role of 5HT3 receptors in mediating the effect of experimental diets on the DMI of the pigs was evaluated by the administration of ondansetron hydrochloride (Tocris, Burlington, NA, USA, #2891), a selective 5-HT3 receptor antagonist, and vehicle (0.9% saline). Following an overnight fast, ondansetron (10 mL; 200 μg/kg in sterile 0.9% saline) [41] and saline (10 mL) were injected into all pigs subcutaneously on days 17 and 20 of the study, respectively at 0700 (Figure 3.1), followed by feeding and recording of the DMI at 0800, 1100, 1400, 1700, 2000, 0200 (next day), and 0800 (next day).

3.3.6. Feed Samples Collection

During the diet preparations, the feed samples (~50 g) were collected from each feed bag and combined for each diet. Afterwards, they were stored at −20 °C, until feed composition analysis.

3.3.7. Meal Test and Blood and Tissue Collection

After an overnight fast (8 h) at week 3, the pigs were allowed to consume their respective diets for 1 h. Blood samples were collected at baseline and then at 60 and 120 min after the meal test from the jugular vein in the supine position into 10 mL serum tubes and 3 mL heparin containing plasma tubes (BD Vacutainer®, Franklin Lakes, NJ, USA). The blood samples were centrifuged at 3000× g for 15 min at 4 °C, and the serum and plasma were separated and stored at −80 °C for further analysis. Following the blood collection at 120 min after the meal test, all pigs were euthanized using the CO2 asphyxiation method. As previously described [38], the hypothalamus, duodenum, jejunum, white adipose tissue, and liver were collected immediately after euthanasia, flushed with distilled water, snap-frozen in liquid nitrogen, and stored at −80 °C for further analyses.

3.3.8. Diet Proximate and Amino Acids Analysis

The dry matter, crude protein, crude fiber, calcium, and phosphorus of the diets and some ingredients contents (i.e., Arg, His, Phe, and whey powder) were analyzed by Servi-Tech laboratory (Dodge City, KS, USA) (Supplemental Table 3A.2), as previously described [38,42,43,44]. The complete amino acids profile of the experimental diets and whey protein concentrate were analyzed [45] by Agricultural Experiment Station Chemical Laboratories (University of Missouri-Columbia, MO, USA) (Supplemental Tables 3A.3 and 3A.4).

3.3.9. Thermal Radiation Analysis

Using a free drawing tool of the FLIR camera software (FLIR Research Studio software), a region of interest was drawn in a rectangular shape on the entire back of the piglets, roughly from shoulders

to the rump of the animal (Supplemental Figure 3A.1), to obtain the dorsal surface body average temperature. The heat loss by thermal radiation (W/m2) was determined using the following equation: σε (Ts4 − Tα4), where σ is the Stefan Boltzmann Constant (5.67 × 10−8 W/m2K4), ε is the thermodynamic emissivity (0.95), Ts is the body surface temperature (kelvin), and Tα is the ambient temperature (kelvin) [46,47].

3.3.10. Plasma Metabolites Analysis

The plasma glucose, triglycerides, and cholesterol concentration were determined by a chemistry analyzer system (CLC 480/BioLis24i, Carolina Liquid Chemistries Corp., Brea, CA), following calibration with a calibrator (Catalogue #: BL-442600, Multi-Analyte calibrator for Synchron CX/LX), using reagents (Carolina Liquid Chemistries Crop, Brea, CA, USA) for glucose (Catalogue #: BL-208), cholesterol (Catalogue #: BL-211), and triglycerides (Catalogue #: BL-213), and recording the absorbance at 340 nm for glucose and at 505 nm for triglycerides and cholesterol.

3.3.11. H&E Staining and Gut Morphology Measurements

Duodenum and jejunum segments were fixed in 10% formaldehyde, coated in paraffin, cut into 5 μm thick sections, and stained with hematoxyl and eosin. Ten well-oriented villi and crypt in each section were used to measure the villus height, villus width, crypt depth, crypt width, and muscle thickness [48] using a BZ-X800E Keyence all-in-one Fluorescence Microscope (BZ-X710, IL, USA), with the images taken with the Keyence BZ-X Viewer (Keyence Co. USA, Itasca, IL, USA) and the analysis performed using the ImageJ software (2 April 2020, https://imagej.nih.gov/ij/download.html).

3.3.12. RNA Isolation and RT-qPCR

RNA isolation and RT-qPCR were performed for glucose transporter 1 (GLUT1), glucokinase (GCK), liver-type phosphofructokinase (PFKL), liver-type pyruvate kinase (PKLR), pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6PC), glucose transporter 2 (GLUT2), lipoprotein lipase (LPL), cluster of differentiation 36 molecule (CD36), fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), hydroxyacyl-CoA dehydrogenase (HADH), sterol regulatory element binding transcription factor 1 (SREBP-1), peroxisome proliferator activated receptor alpha (PPARα), and PPARG coactivator 1 alpha (PGC1α) in the liver, following our published procedures [38,49,50]. The quality of isolated RNA was measured by a NanoDrop ND-1000 spectrophotometer (Thermo Fisher, Waltham, MA, USA). The complementary cDNA was synthesized by a thermoscycler (T100TM Thermal Cycler, Bio-Rad, Hercules, CA, USA), and then a CFX96 real-time PCR detection system (Bio-Rad, Hercules, CA, USA) was used to measure the mRNA abundance of the target genes through real-time quantitative PCR (qPCR), followed by a melt curve, as previously described [38,49,50]. The primers used are shown in Supplemental Table 3A.5 and were obtained from previous publications [51,52,53,54,55,56,57,58]. β-actin was used as a housekeeping gene, and the relative mRNA abundance of the target genes was calculated by the $2^{-\Delta\Delta CT}$ method.

3.3.13. Immunoblot Analysis

Western blot was conducted in hypothalamus for tryptophan hydroxylase 2 (TPH2), jejunum for TPH2, sodium/glucose cotransporter 1 (SGLT1), and glucose transporter 2 (GLUT2), duodenum for SGLT1 and GLUT2, liver for carnitine palmitoyltransferase I α (CPT1α) and GLUT2, and white adipose tissues for CPT1α (Supplemental Table 3A.6), as previously described [59,60]. The protein bands were captured using a ChemiDoc XR imaging system (Bio-Rad Laboratories Inc., CA, USA). Image Lab software (Version 6.0.1, Bio-Rad Laboratories Inc., CA, USA) was used for applying densitometry. In order to determine the relative amount of protein abundance in the sample, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control.

3.3.14. Plasma Insulin

The plasma insulin concentration was determined using a porcine ELISA kit, according to the manufacturer's instructions (Mercodia Porcine Insulin ELISA; Mercodia AB; Uppsala, Sweden). The optical density was read at a 450 nm wavelength using an Epoch microplate spectrophotometer (BioTek® Instruments, Inc. Highland Park, VT). The intra-assay coefficient of variation (CV) was 4.8%. The insulin resistance indicator, HOMA-IR, was calculated, as previously described [61]. The baseline blood glucose concentrations (G0, milligrams per deciliter) and the fasting blood insulin concentrations (I0, micro-international units per milliliter) were used to calculate HOMA-IR, using the following formula: (G0 × I0)/2430.

3.3.15. Statistical Analysis

The final body weight, heart girth, body length, wither size, ADG, ADMI, ADPI, G:F, G:P, plasma cholesterol and triglycerides, gut histomorphometry, qPCR, ELISA, and western blot data were analyzed by the GLM procedure (IBM SPSS Statistics Version 23, Armonk, NY, USA), followed by a post hoc Dunnett's test (IBM SPSS Statistics Version 23, Armonk, NY, USA). The plasma glucose concentration distribution was not normal at 60 min after the meal. Therefore, an inverse distribution function (IDF-normal) was used to normalize the data at this time point. The mixed procedure was used for the analysis of blood glucose, DMI, BW, rectal temperature, and thermal radiation, with diet, time, and the interaction of diet and time as fixed variables and pigs as a random variable. As previously described [42,43,50], the covariance structure of repeated measurements was modeled for each variable based on the smallest levels of fit statistics for corrected Akaike's Information Criterion and Bayesian Information Criterion. Before statistical analysis of all the data, the outlier test was performed in SPSS (IBM SPSS Statistics Version 23, Armonk, NY, USA), which is based on the Interquartile Rule. $p \leq 0.05$ and $0.05 < p \leq 0.10$ were considered as significant and trend, respectively.

3.4. Results

3.4.1. Feed Intake, Body Weight, and Growth Measurements

Overall, except for the G:F ratio, the effect of diet on the initial BW, final BW, ADG, ADMI, ADPI, G:P ratio, heart girth, wither height, and body length was significant (Table 3.1). NBW-T0 had a greater initial BW, final BW, ADG, ADMI, ADPI, heart girth, wither height, and body length, compared to LBW-T0 (~54, 30, 22, 31, 27, 10, 8, and 11%, respectively). Moreover, ADPI was lower in LBW-T0.8 (11.7%), compared to LBW-T0.

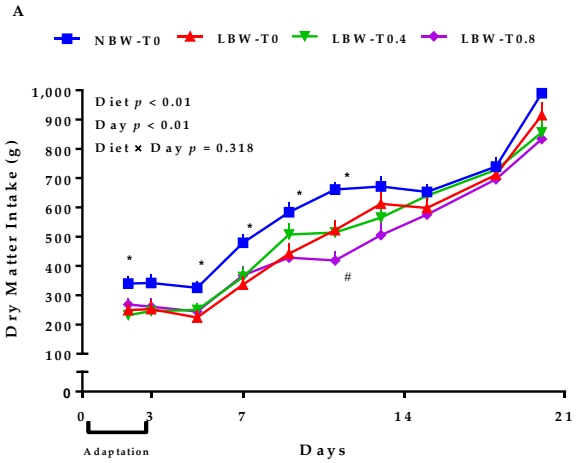
Table 3.1. Effect of dietary L-tryptophan supplementation on the growth and feed efficiency of low-birthweight neonatal pigs.

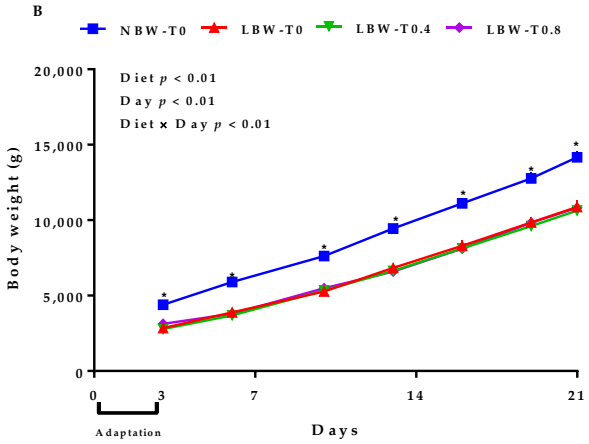
Measurements	Diets[1]				SEM[2]	*p*-Value
	NBW-T0	LBW-T0	LBW-T0.4	LBW-T0.8		
Initial BW[3], g	4393*	2853	2783	3123	145	<0.01
Final BW, g	14,172*	10,877	10,634	10,818	335	<0.01
ADG[3], g/day	543*	445	436	427	12	<0.01
ADMI[3], g/day	554*	422	421	399	14	<0.01
ADPI[3], g/day	119*	94	99	83*	2.7	<0.01
G:F[3], g/g	1.00	1.05	0.97	1.07	0.01	0.26
G:P[3], g/g	4.65	4.77	4.30	5.14	0.10	0.02
Heart girth, cm	55.12*	50.00	50.42	51.37	0.53	<0.01
Wither height, cm	34.75*	32.00	31.14	31.25	0.46	<0.01
Body length, cm	54.00*	48.62	46.42	47.37	0.66	<0.01

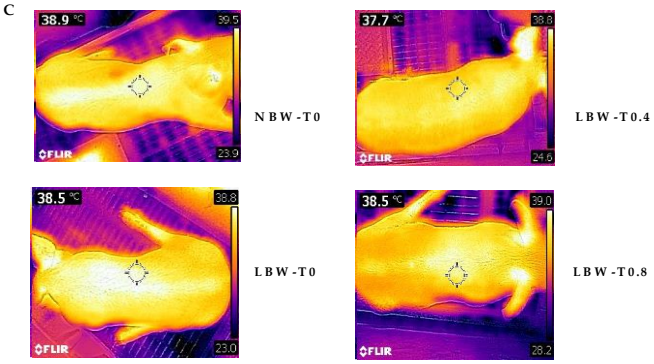
[1]NBW-T0, normal-birthweight piglets fed a basal diet without supplemented L-tryptophan (Trp); LBW-T0, low-birthweight piglets fed a basal diet without supplemented Trp; LBW-T0.4, low-birthweight piglets fed a basal diet supplemented with 0.4% Trp; and LBW-T0.8, low-birthweight piglets fed a basal diet supplemented with 0.8% Trp. The values are the means. n = 8 for NBW-T0, LBW-T0, and LBW-T0.8, and n = 7 for LBW-T0.4. 2 SEM: standard errors of means. [3] BW: body weight; ADG: average daily gain; ADMI: average dry matter intake; ADPI: average daily protein intake; G:F: gain:feed; G:P: gain:protein. * $P \leq 0.05$ vs. LBW-T0.

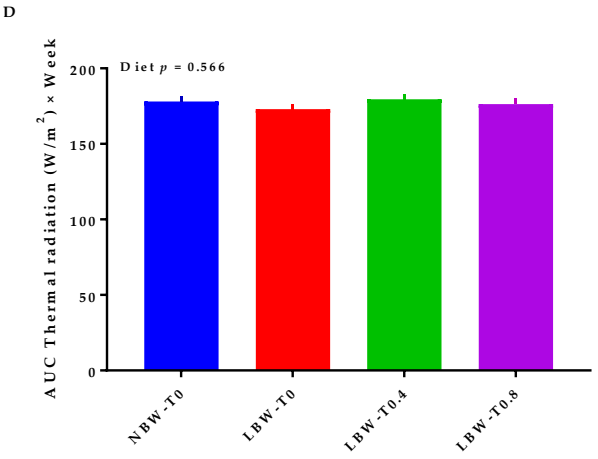
Overall, the effect of diet and day on daily DMI was significant (Figure 3.2A). Compared to LBW-T0, NBW-T0 had a higher (~26–45%) DMI on days 2, 5, 7, 9, and 11 of the study (Figure 3.2A).

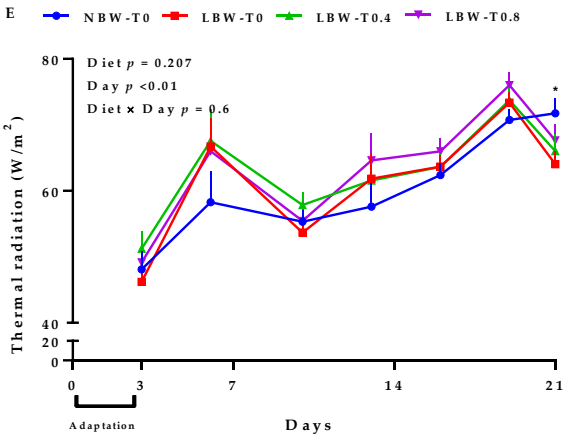
LBW-T0.8 tended to have a lower DMI, compared to LBW-T0, on day 11 (Figure 3.2A). The 1-h meal test DMI for NBW-T0, LBW-T0, LBW-T0.4, and LBW-T0.8 was 154 ± 10 g, 126 ± 19 g, 125 ± 18 g, and 123 ± 17 g, respectively. As expected, the DMI of NBW-T0 vs. LBW-T0 was significantly different ($p < 0.05$), but no differences in DMI were seen among the other groups. The effect of diet on BW was significant, with 9310, 6838, 6687, and 6787 g for NBW-T0, LBW-T0, LBW-T0.4, and LBW-T0.8, respectively (Figure 3.2B). Compared to LBW-T0, NBW-T0 had a higher BW throughout the study (~30–54%). No differences in the BW of pigs were detected, when LBW-T0 was compared with LBW-T0.4 or LBW-T0.8 (Figure 3.2B).


A
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
Diet p < 0.01
Day p < 0.01
Diet × Day p = 0.318
Dry Matter Intake (g)
Adaptation
Days


B
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
Diet p < 0.01
Day p < 0.01
Diet × Day p < 0.01
Body weight (g)
Adaptation
Days


C
NBW-T0
LBW-T0.4
LBW-T0
LBW-T0.8


D
Diet p = 0.566
AUC Thermal radiation (W/m2) × Week
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8


E
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
Diet p = 0.207
Day p <0.01
Diet × Day p = 0.6
Thermal radiation (W/m2)
Adaptation
Days

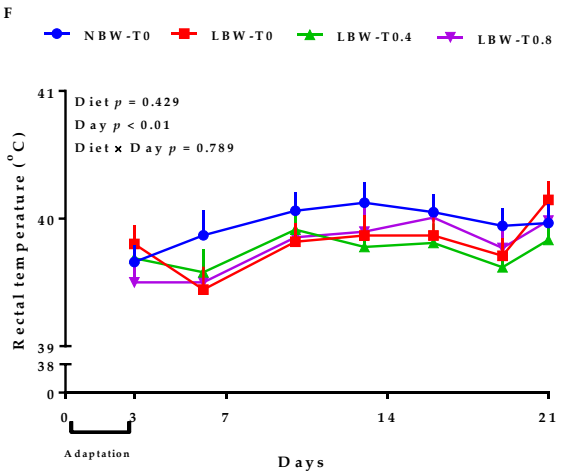

F
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
Diet p = 0.429
Day p < 0.01
Diet × Day p = 0.789
Rectal temperature (°C)
Adaptation
Days

Figure 3.2. Effect of dietary L-tryptophan supplementation on dry matter intake, body weight, thermal radiation, and area under the curve (AUC) for the thermal radiation and rectal temperature of low-birthweight neonatal pigs.

(A) dry matter intake, (B) body weight, (C) representative thermal images, (D) area under the curve (AUC) thermal radiation, (E) thermal radiation, and (F) rectal temperature. NBW-T0, normal-birthweight piglets fed a basal diet without supplemented L-tryptophan (Trp); LBW-T0, low-birthweight piglets fed a basal diet without supplemented Trp; LBW-T0.4, low-birthweight piglets fed a diet supplemented with 0.4% Trp; and LBW-T0.8, low-birthweight piglets fed a diet supplemented with 0.8% Trp. n = 8 for NBW-T0, LBW-T0, and LBW-T0.8, and n = 7 for LBW-T0.4. * $P \leq 0.05$ vs. LBW-T0; $^{\#}$ $0.05 < P \leq 0.1$ vs. LBW-T0. The values are the means ± SEM.

3.4.2. Thermal Radiation

Compared to LBW-T0, no differences in the thermal radiation, the area under the curve (AUC) for thermal radiation, and the rectal temperature were detected among the treatments (Figure 3.2C–F).

3.4.3. Gut Histomorphology

The histomorphometry data showed a tendency toward a greater villus height to crypt depth ratio in NBW-T0, compared to LBW-T0 in duodenum (Table 3.2; Figure 3.3). Compared to LBW-T0, the crypt depth tended to be higher in LBW-T0.8 in jejunum. No differences among the groups were observed for the villus height, villus width, crypt width, and muscle thickness in both duodenum and jejunum (Table 3.2; Figure 3.3).

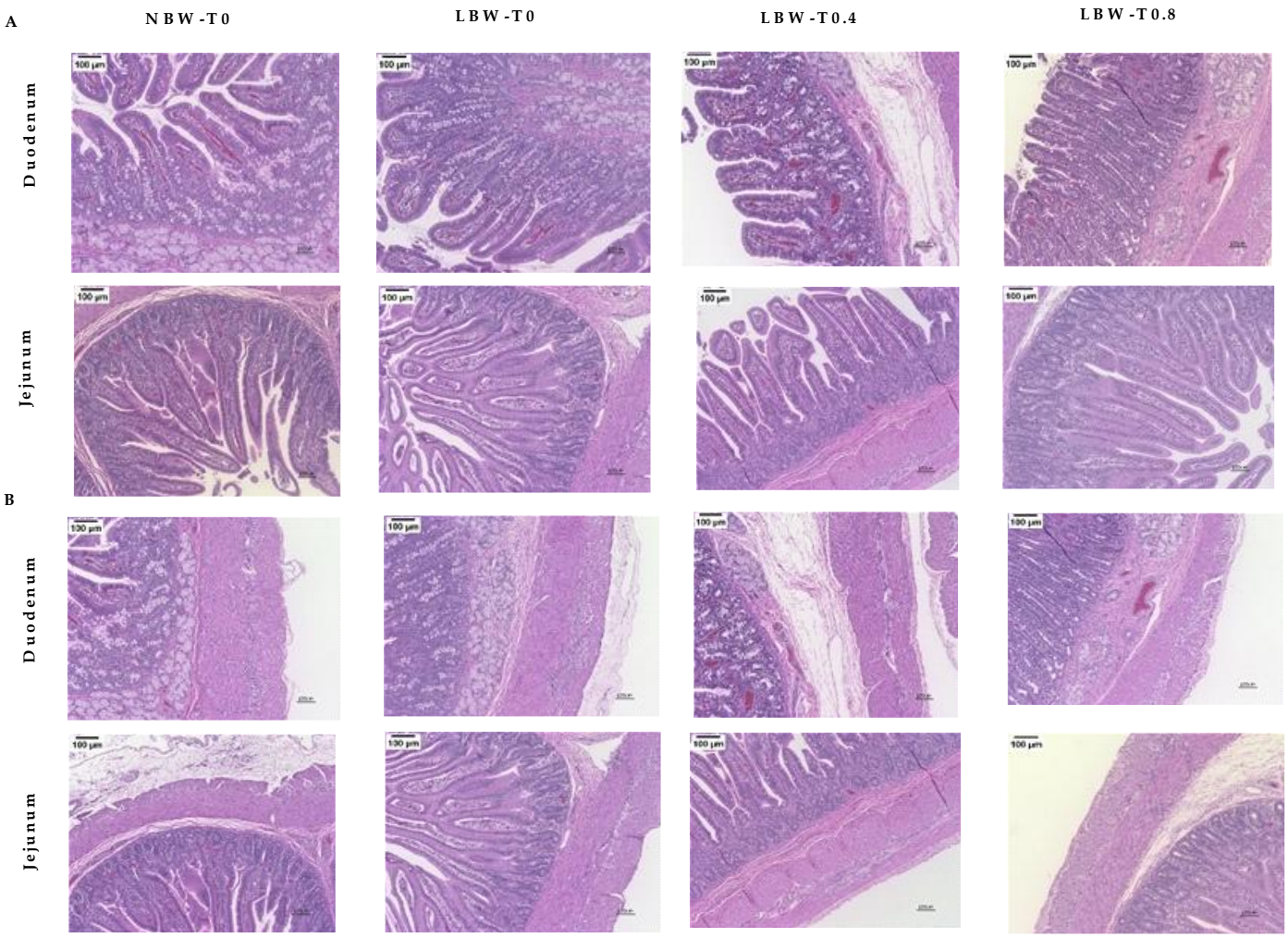


Figure 3.3. Effect of dietary L-tryptophan supplementation on the morphology and development of the duodenum and jejunum in low-birthweight neonatal pigs.

Representative hematoxylin and eosin-stained sections (×10 magnification) micrograph of the duodenum and jejunum for (A) villi and (B) muscle. NBW-T0, normal-birthweight piglets fed a basal diet without supplemented L-tryptophan (Trp); LBW-T0, low-birthweight piglets fed a basal diet without supplemented Trp; LBW-T0.4, low-birthweight piglets fed a diet supplemented with 0.4% Trp; and LBW-T0.8, low-birthweight piglets fed a diet supplemented with 0.8% Trp.

Table 3.2. Effect of dietary L-tryptophan supplementation on the intestinal morphology of low-birthweight neonatal pigs.

Measurements	Diets[1]				SEM[2]	*p*-Value
	NBW-T0	LBW-T0	LBW-T0.4	LBW-T0.8		
Duodenum						
Villi height, μm	562	497	585	530	20	0.44
Villi width, μm	138	124	138	123	3	0.13
Crypt depth, μm	229	224	230	198	8	0.54
Crypt width, μm	64	55	60	55	2	0.12
Muscle thickness, μm	385	376	447	386	17	0.46
Villi height:Crypt depth, μm:μm	2.90 #	2.03	2.57	2.69	0.13	0.13
Jejunum						
Villi height, μm	619	595	589	594	28	0.98
Villi width, μm	121	106	118	125	3	0.23
Crypt depth, μm	200	211	221	256#	8	0.02
Crypt width, μm	48	49	53	55	1	0.24
Muscle thickness, μm	323	367	410	463	22	0.12
Villi height:Crypt depth, μm:μm	2.82	3.03	2.65	2.50	0.12	0.50

[1]NBW-T0, normal-birthweight piglets fed a basal diet without supplemented L-tryptophan (Trp); LBW-T0, low-birthweight piglets fed a basal diet without supplemented Trp; LBW-T0.4, low-birthweight piglets fed a diet sup-plemented with 0.4% Trp; and LBW-T0.8, low-birthweight piglets fed a diet supplemented with 0.8% Trp. The values are the means. n = 4–5 for NBW-T0, LBW-T0.4, and LBW-T0.8, and n = 3–5 for LBW-T0. [2] SEM: standard errors of means. # $0.05 < P \leq 0.1$ vs. LBW-T0.

3.4.4. 5HT3 Receptors Blockade with Ondansetron

Overall, the effect of the drug on DMI was significant ($p < 0.01$; Figure 3.4), showing a lower DMI for ondansetron vs. vehicle (466 ± 14 vs. 518 ± 14 g, respectively), and the overall effect of diet on DMI tended to be significant ($p = 0.083$; Figure 3.4), showing the lowest DMI for LBW-T0.8. The DMI for NBW-T0, LBW-T0, LBW-T0.4, and LBW-T0.8 were 540 ± 26 g, 505 ± 26 g, 477± 27 g, and 445 ± 26 g, respectively. Relative to the vehicle, the ondansetron decreased the DMI by ~8–16% in NBW-T0 and 16–20% in LBW-T0.8 during a 24 h period (Figure 3.4A,D), while the ondansetron reduced the DMI by 14% in LBW-T0 only during the first 6 h (Figure 3.4B). No

differences in DMI were detected when the vehicle and ondansetron were compared for LBW-T0.4 (Figure 3.4C).

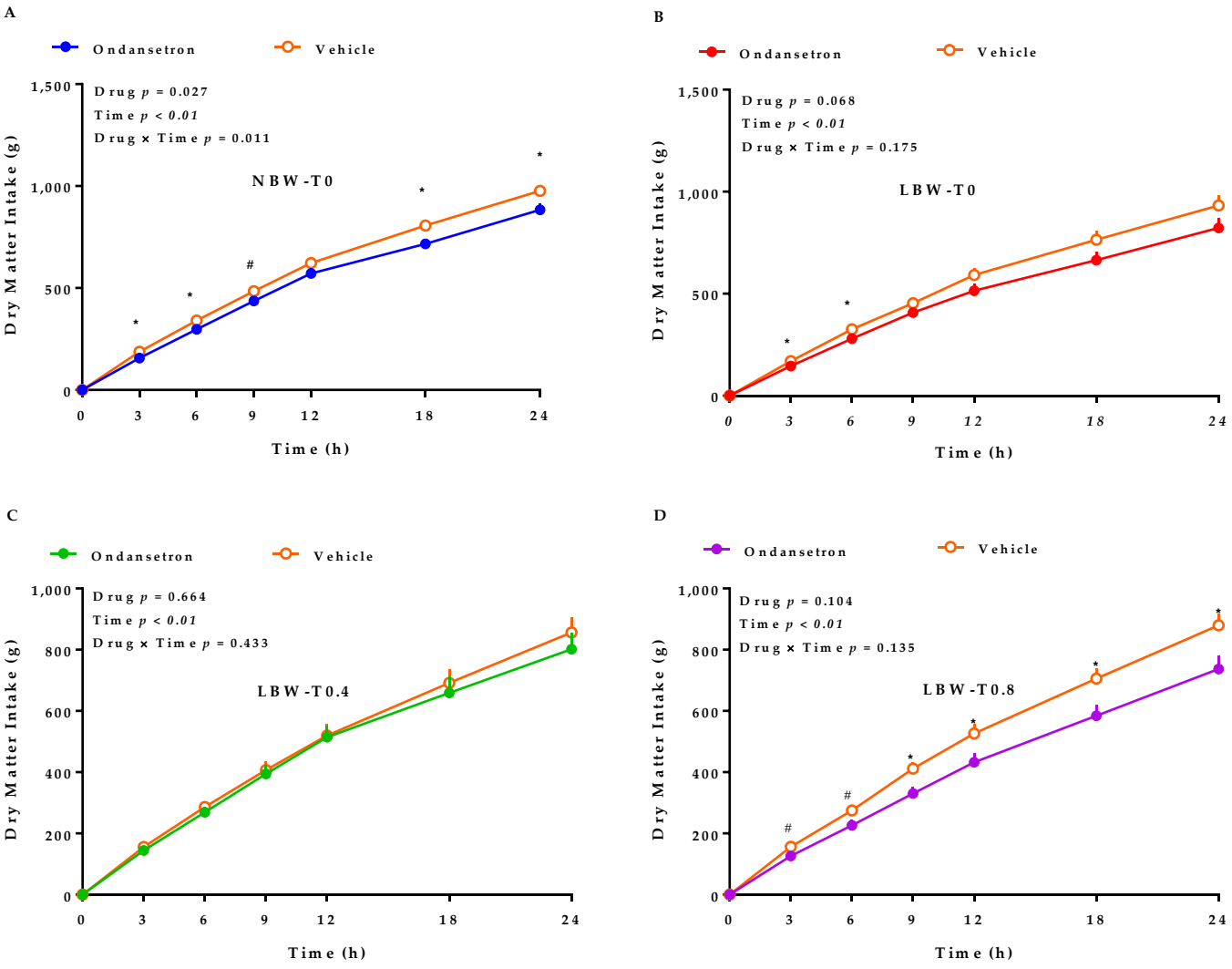


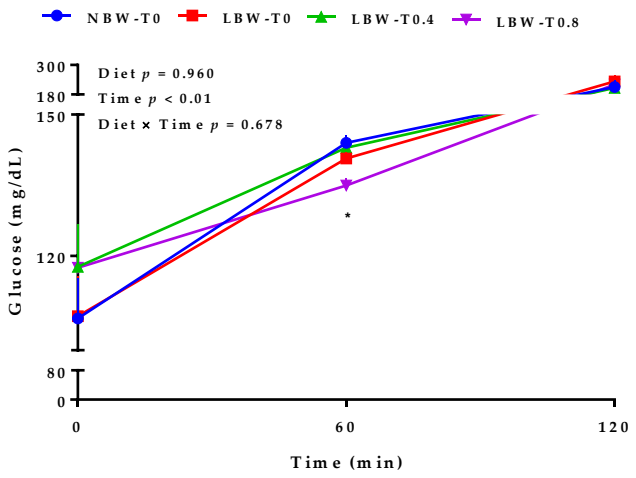
Figure 3.4. Effect of saline or ondansetron injection on the dry matter intake of low-birthweight neonatal pigs fed with milk-based diets supplemented with L-tryptophan.

(A) NBW-T0, normal-birthweight piglets fed a basal diet without supplemented L-tryptophan (Trp), (B) LBW-T0, low-birthweight piglets fed a basal diet without supplemented Trp, (C) LBW-T0.4, low-birthweight piglets fed a diet supplemented with 0.4% Trp, and (D) LBW-T0.8, low-birthweight piglets fed a diet supplemented with 0.8% Trp. All pigs were injected either with saline or the drug (ondansetron, 200 μg × kg−1, SC). The p values for the overall model effect for the drug, time, diet, drug × time, drug × diet, time × diet and drug × time × diet for dry matter intake were <0.01, <0.01, 0.083, <0.01, 0.470, 0.108, and 0.707, respectively. n = 8 for NBW-T0, LBW-T0, and LBW-T0.8, and n = 7 for LBW-T0.4. * $P \leq 0.05$ saline vs. ondansetron within each diet; $^{\#}$ $0.05 < P \leq 0.1$ saline vs. ondansetron within each diet. The values are the means ± SEM.

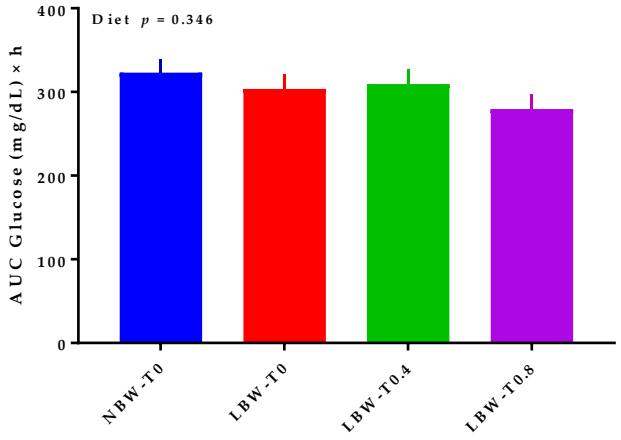
3.4.5. Blood Glucose, Triglycerides, Cholesterol, and Insulin

No differences in blood glucose were observed among the treatments at 0 and 120 min following the meal test (Figure 3.5A). However, LBW-T0.8 had a lower (~4%) blood glucose than LBW-T0 at 60 min after the meal challenge (Figure 3.5A). The AUC for blood glucose did not change across groups (Figure 3.5B). Likewise, the plasma insulin at 0 and 60 min after the meal challenge (Figure 3.5C) and HOMA-IR was not different across groups. Relative to LBW-T0, the plasma triglyceride concentration was decreased in LBW-T0.4 and LBW-T0.8 by 57% and 51%, respectively (Figure 3.5D). The total cholesterol did not differ across groups (Figure 3.5E)

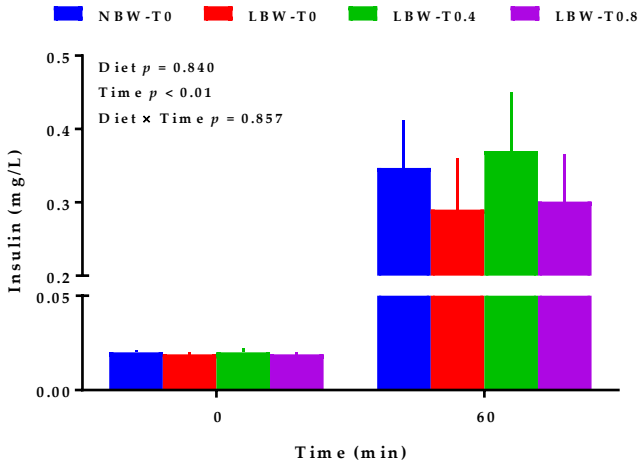
A


NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
Diet p = 0.960
Time p < 0.01
Diet × Time p = 0.678
Glucose (mg/dL)
300
180
150
120
80
0
0
60
120
Time (min)
*

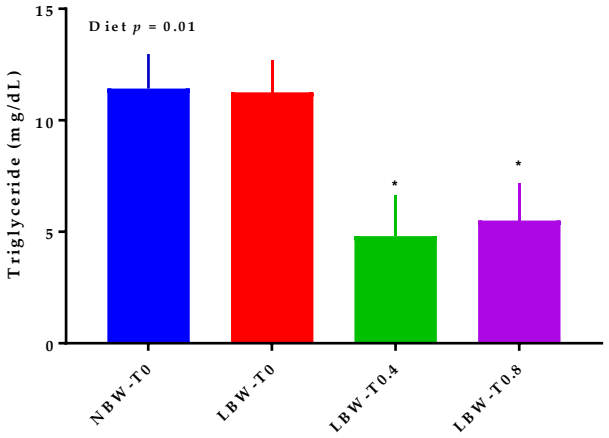
B


Diet p = 0.346
AUC Glucose (mg/dL) × h
400
300
200
100
0
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8

C


NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
Diet p = 0.840
Time p < 0.01
Diet × Time p = 0.857
Insulin (mg/L)
0.5
0.4
0.3
0.2
0.05
0.00
0
60
Time (min)

D


Diet p = 0.01
Triglyceride (mg/dL)
15
10
5
0
*
*
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8

E

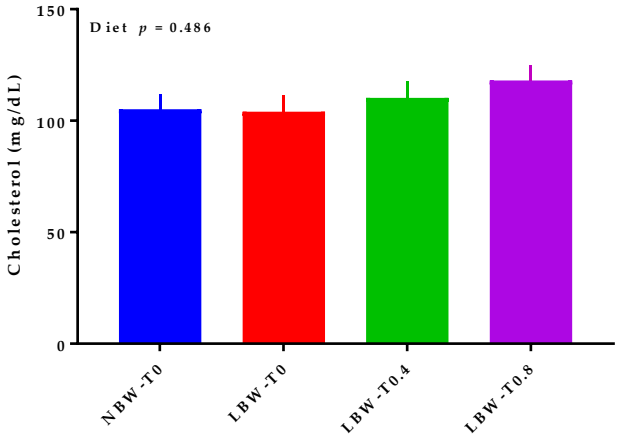

Diet p = 0.486
Cholesterol (mg/dL)
150
100
50
0
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8

Figure 3.5. Effect of dietary L-tryptophan supplementation on plasma metabolite concentrations in low-birthweight neonatal pigs.

(A) glucose, (B) area under the curve (AUC) for glucose, (C) insulin, (D) triglyceride, and (E) cholesterol. NBW-T0, normal-birthweight piglets fed a basal diet without supplemented L-tryptophan (Trp); LBW-T0, low-birthweight piglets fed a basal diet without supplemented Trp; LBW-T0.4, low-birthweight piglets fed a diet supplemented with 0.4% Trp; and LBW-T0.8, low-birthweight piglets fed a diet supplemented with 0.8% Trp. n = 8 for NBW-T0, LBW-T0, and LBW-T0.8, and n = 7 for LBW-T0.4. * $P \leq 0.05$ vs. LBW-T0. The values are the means ± SEM.

3.4.6. The mRNA Abundance of Key Molecules of Glucose and Lipid Metabolism in the Liver

Compared to LBW-T0, the mRNA abundance of GLUT1 was higher in NBW-T0 (Figure 3.6A). Relative to LBW-T0, the mRNA abundance of GCK was greater in LBW-T0.8 (Figure 3.6B). The mRNA expression of liver GLUT2 was lower, and PEPCK tended to be lower in LBW-T0.8, compared to LBW-T0 (Figure 3.6H,F). No differences among treatments were detected for the mRNA abundance of PFKL (Figure 3.6C), PKLR (Figure 3.6D), PC (Figure 3.6E), and G6PC (Figure 3.6G).

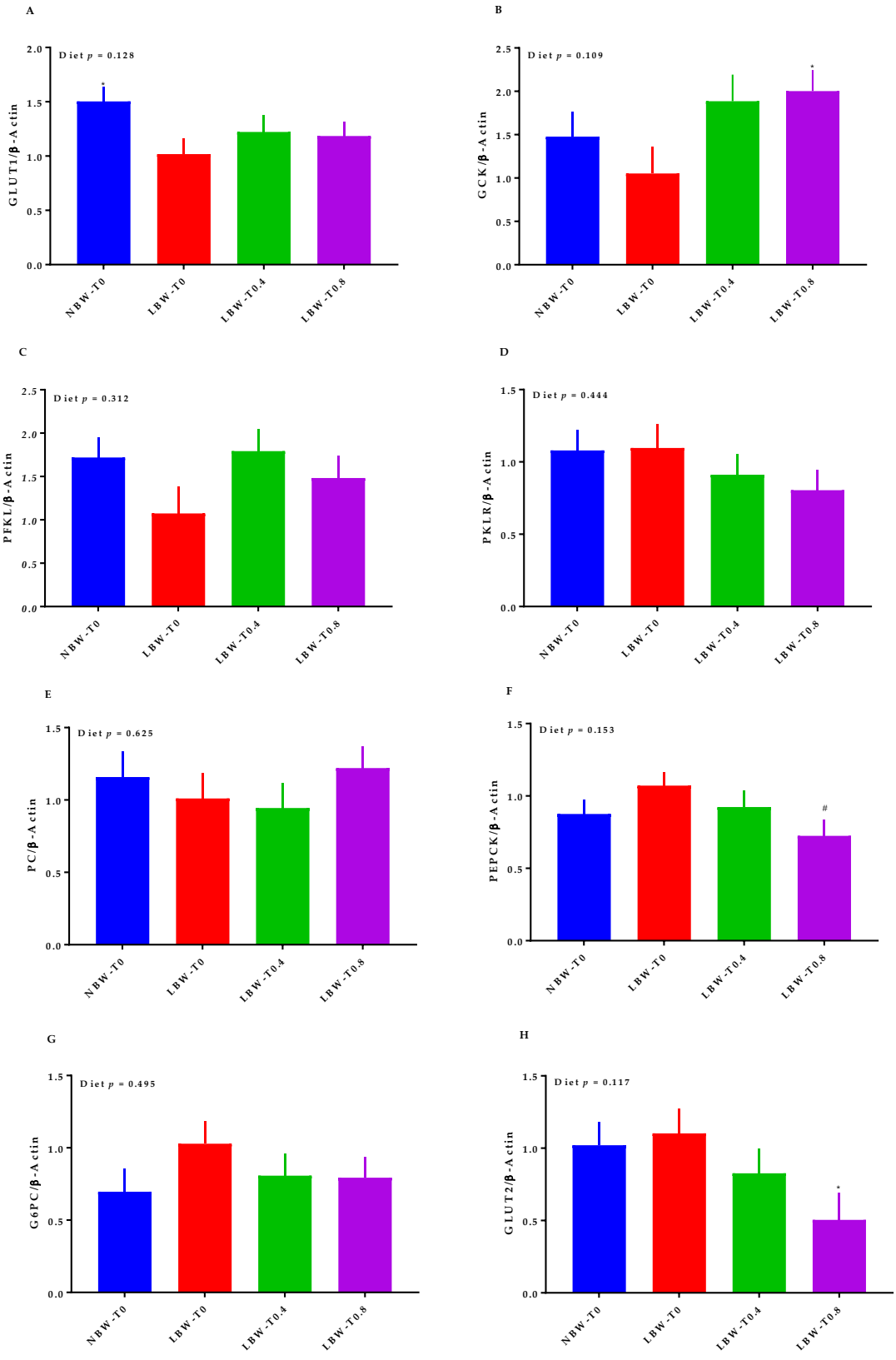

A
Diet p = 0.128
GLUT1/β-Actin
2.0
1.5
1.0
0.5
0.0
*
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
B
Diet p = 0.109
GCK/β-Actin
2.5
2.0
1.5
1.0
0.5
0.0
*
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
C
Diet p = 0.312
PFKL/β-Actin
2.5
2.0
1.5
1.0
0.5
0.0
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
D
Diet p = 0.444
PKLR/β-Actin
1.5
1.0
0.5
0.0
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
E
Diet p = 0.625
PC/β-Actin
1.5
1.0
0.5
0.0
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
F
Diet p = 0.153
PEPCK/β-Actin
1.5
1.0
0.5
0.0
#
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
G
Diet p = 0.495
G6PC/β-Actin
1.5
1.0
0.5
0.0
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
H
Diet p = 0.117
GLUT2/β-Actin
1.5
1.0
0.5
0.0
*
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8

Figure 3.6. Effect of dietary L-tryptophan supplementation on the liver mRNA abundance of glucose metabolism markers in low-birthweight neonatal pigs.

(A) glucose transporter 1 (GLUT 1), (B) glucokinase (GCK), (C) phosphofructokinase, liver-type (PFKL), (D) pyruvate kinase, liver, and RBC (PKLR), (E) pyruvate carboxylase (PC), (F) phosphoenolpyruvate carboxykinase (PEPCK), (G) glucose-6-phosphatase catalytic (G6PC), and (H) glucose transporter 2 (GLUT2). NBW-T0, normal-birthweight piglets fed a basal diet without supplemented L-tryptophan (Trp); LBW-T0, low-birthweight piglets fed a basal diet without supplemented Trp; LBW-T0.4, low-birthweight piglets fed a diet supplemented with 0.4% Trp; and LBW-T0.8, low-birthweight piglets fed a diet supplemented with 0.8% Trp. n = 8 for NBW-T0, LBW-T0, and LBW-T0.8, and n = 7 for LBW-T0.4. * $P \leq 0.05$ vs. LBW-T0; $^{\#}$ $0.05 < P \leq 0.1$ vs. LBW-T0. The values are the means ± SEM.

The effect of diet on the mRNA abundance of liver ACC and HADH was significant ($p < 0.05$, Figure 3.7D,E). The mRNA abundance of ACC was lower in LBW-T0.4 and LBW-T0.8, compared to LBW-T0 (Figure 3.7D). The transcript of liver HADH was greater in LBW-T0.4, compared to LBW-T0 (Figure 3.7E). The effect of diet on FAS (Figure 3.7C) and the SREBP-1 (Figure 3.7F) transcript tended to be significant, with a lower mRNA abundance for these markers in LBW-T0.4 and LBW-T0.8 than LBW-T0. There were no significant differences across treatments for the mRNA abundance of LPL (Figure 3.7A), CD36 (Figure 3.7B), and PPARα (Figure 3.7G).

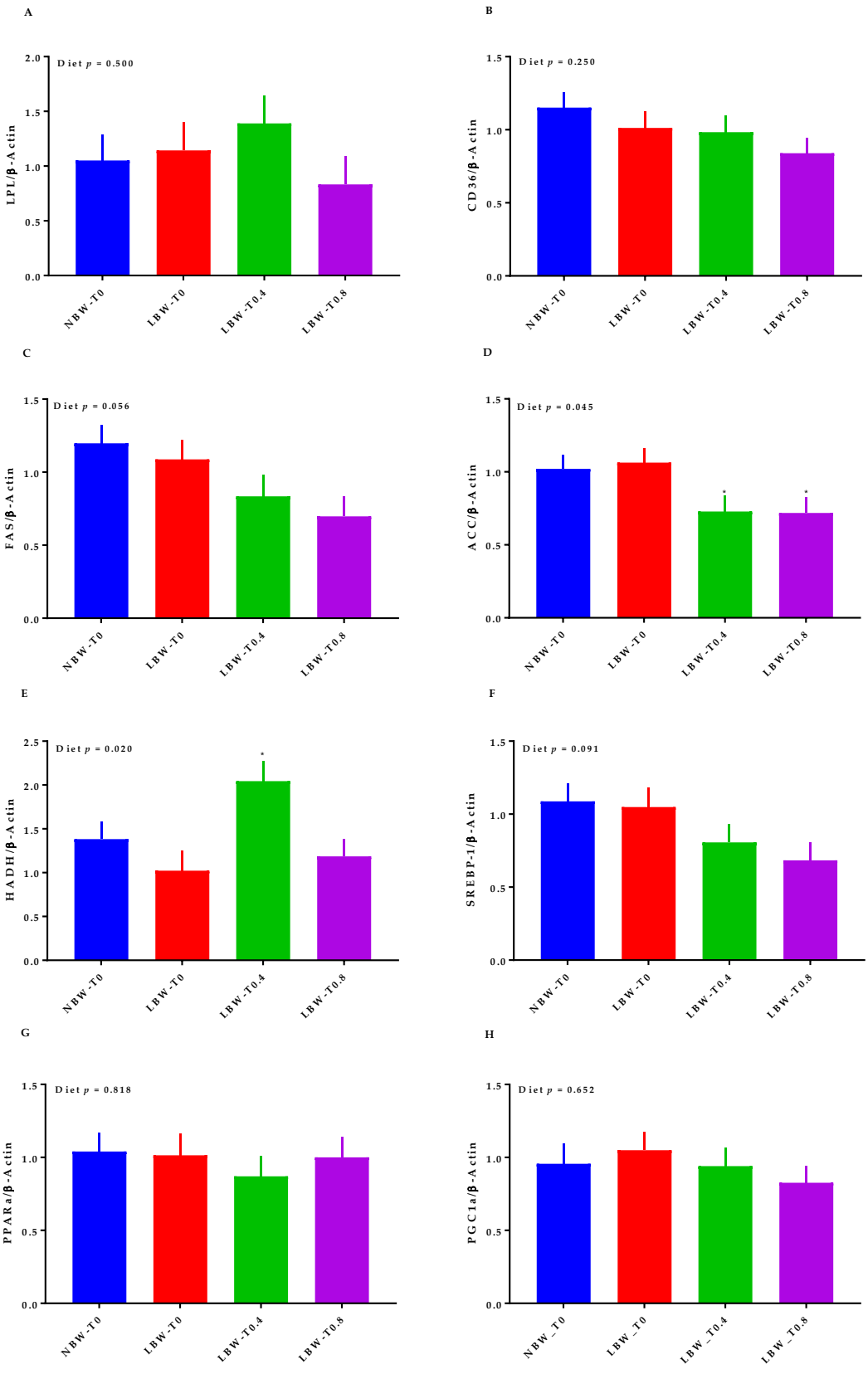

A
Diet p = 0.500
LPL/β-Actin
2.0
1.5
1.0
0.5
0.0
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
B
Diet p = 0.250
CD36/β-Actin
1.5
1.0
0.5
0.0
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
C
Diet p = 0.056
FAS/β-Actin
1.5
1.0
0.5
0.0
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
D
Diet p = 0.045
ACC/β-Actin
1.5
1.0
0.5
0.0
*
*
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
E
Diet p = 0.020
HADH/β-Actin
2.5
2.0
1.5
1.0
0.5
0.0
*
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
F
Diet p = 0.091
SREBP-1/β-Actin
1.5
1.0
0.5
0.0
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
G
Diet p = 0.818
PPARa/β-Actin
1.5
1.0
0.5
0.0
NBW-T0
LBW-T0
LBW-T0.4
LBW-T0.8
H
Diet p = 0.652
PGC1a/β-Actin
1.5
1.0
0.5
0.0
NBW_T0
LBW_T0
LBW_T0.4
LBW_T0.8

Figure 3.7. Effect of dietary L-tryptophan supplementation on the liver mRNA abundance of lipid metabolism markers in low-birthweight neonatal pigs.

(A) lipoprotein lipase (LPL), (B) cluster of differentiation 36 molecule (CD36), (C) fatty acid synthase (FAS), (D) acetyl-CoA carboxylase alpha (ACC), (E) hydroxyacyl-CoA dehydrogenase (HADH), (F) sterol regulatory element-binding transcription factor 1 (SREBP-1), (G) peroxisome proliferator activated receptor alpha (PPARα), and (H) PPARG coactivator 1 alpha (PGC1α). NBW-T0, normal-birthweight piglets fed a basal diet without supplemented L-tryptophan (Trp); LBW-T0, low-birthweight piglets fed a basal diet without supplemented Trp; LBW-T0.4, low-birthweight piglets fed a diet supplemented with 0.4% Trp; and LBW-T0.8, low-birthweight piglets fed a diet supplemented with 0.8% Trp. n = 8 for NBW-T0, LBW-T0, and LBW-T0.8, and n = 7 for LBW-T0.4. * $P \leq 0.05$ vs. LBW-T0. The values are the means ± SEM.

3.4.7. The Protein Abundance of Key Molecules of Glucose and Lipid Metabolism in the Hypothalamus, Liver, Jejunum, Duodenum, and White Adipose Tissue

The protein abundance of SGLT1 tended to be lower in LBW-T0.4, compared to LBW-T0, in the jejunum (Figure 3.8D). Moreover, relative to LBW-T0, the GLUT2 protein abundance tended to be lower in LBW-T0.4 and LBW-T0.8 in the liver (Figure 3.8E). Pigs in the NBW-T0 group had a lower protein abundance of CPT1α in the liver than in LBW-T0 (Figure 3.8H). The protein abundance of TPH2 in the hypothalamus and jejunum (Figure 3.8A,B), SGLT1 in the duodenum (Figure 3.8C), GLUT2 in the duodenum and jejunum (Figure 3.8F,G), and CPT1α in white adipose tissue (Figure 3.8I) were not different across dietary treatments. The full-length immunoblots for all the above markers are given in Supplemental Figure 3A.2.

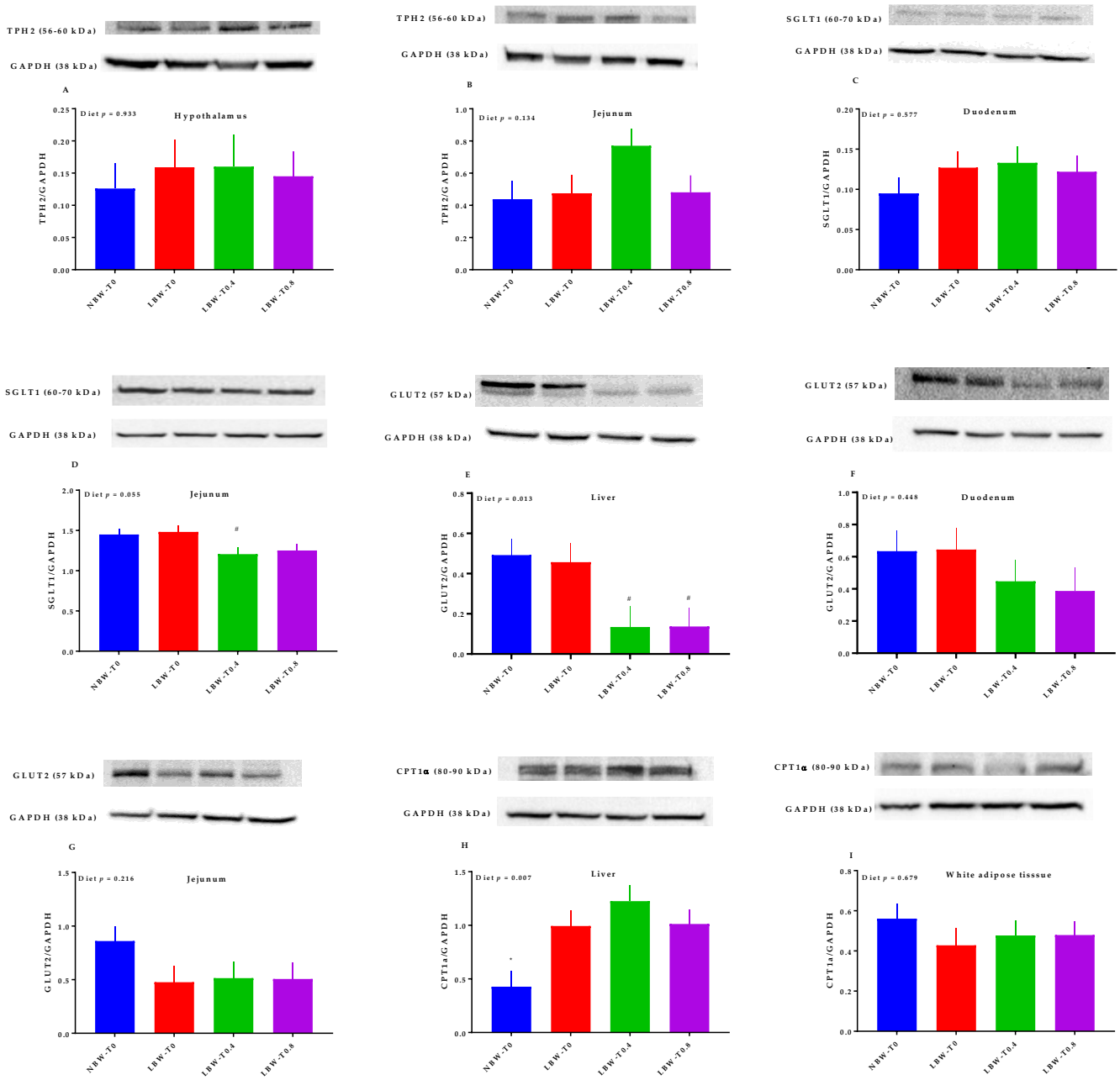


Figure 3.8. Effect of dietary L-tryptophan supplementation on the relative protein abundance of feed intake and glucose and lipid metabolism markers in the hypothalamus, jejunum, duodenum, liver, and white adipose tissue.

(A) tryptophan hydroxylase 2 (TPH2) in the hypothalamus, (B) TPH2 in the jejunum, (C) sodium/glucose cotransporter 1 (SGLT1) in the duodenum, (D) SGLT1 in the jejunum, (E) glucose transporter-2 (GLUT2) in the liver, (F) GLUT2 in the duodenum, (G) GLUT2 in the jejunum, (H) carnitine palmitoyltransferase I α (CPT1α) in the liver, and (I) CPT1α in white adipose tissue. NBW-T0, normal-birthweight piglets fed a basal diet without supplemented L-tryptophan (Trp); LBW-T0, low-birthweight piglets fed a basal diet without supplemented Trp; LBW-T0.4, low-birthweight piglets fed a diet supplemented with 0.4% Trp; and LBW-T0.8, low-birthweight piglets fed a diet supplemented with 0.8% Trp. n = 8 for NBW-T0, LBW-T0, and LBW-T0.8, and n = 7 for LBW-T0.4. * $P \leq 0.05$ vs. LBW-T0; $^{\#}$ $0.05 < P \leq 0.1$ vs. LBW-T0. The values are the means ± SEM.

3.5. Discussion

LBW is not only associated with health complications in the long term [5,6,7,8], but it is also linked with glucose and lipid metabolism disturbances in the short term [9,10]. Nutrition is crucial for the optimal growth and health of LBW during the fetal and postnatal periods [8]. Dietary Trp decreases the serum and hepatic lipids in rodents and chickens [23,24,25] and suppresses the hyperglycemia in rodents [22,26]. Further, Trp is a precursor of serotonin that is involved in feed intake regulation. Whether dietary Trp affects the glucose and lipid metabolism and influences the feeding behavior through peripheral serotonin in LBW neonates has not been elucidated. The objective of this study was to assess: (1) the effect of dietary Trp on lipid and glucose metabolism in a LBW piglet model, and (2) the role of peripheral 5HT3 receptors on feed intake regulation in LBW pigs fed with Trp-enriched diets. Our study generated several key findings: (1) LBW-T0.8 decreased plasma glucose at 60 min after a meal test, which is indicative of the role of Trp in reducing blood glucose. This might be explained by the increased glycolysis (increased hepatic mRNA abundance of GCK), decreased gluconeogenesis (tending to decrease the PEPCK transcript in the liver), and reduced hepatic glucose efflux (reduced hepatic GLUT2 gene and protein abundance) in the LBW-T0.8

group. (2) The blood glucose concentration did not change in LBW-T0.4, and the protein expression of SGLT1 in the jejunum tended to be decreased and GLUT2 in the liver was decreased in this group, suggestive of a reduced absorption of glucose in the gut and glucose efflux in the liver in this group. (3) Both LBW-T0.4 and LBW-T0.8 reduced the plasma triglyceride concentration. These might be due to the reduced hepatic lipogenesis in both groups (reduced mRNA abundance of hepatic ACC) and increased hepatic lipolysis in LBW-T0.4 (increased mRNA abundance of hepatic HADH). (4) Blocking the 5-HT3 receptor with ondansetron reduced the DMI in all groups, which is suggestive of the possible role of peripheral serotonin in the regulation of feeding behavior. (5) Ondansetron decreased the DMI transiently (6 h by 14%) in LBW-T0, but that reduced the DMI throughout the day (24 h by 16–20%) in LBW-T0.8. Additionally, ondansetron specifically reduced the DMI in the NBW-T0 group. These data are suggestive of a higher serotonergic signaling in the LBW-T0.8 and NBW-T0 groups, compared to LBW-T0. Altogether, milk replacers enriched in Trp improved the hepatic lipid and glucose metabolism by reducing the lipogenesis, gluconeogenesis, and glucose efflux and increasing the lipolysis and glycolysis. Peripheral serotonin is likely involved in the regulation of feeding behavior, particularly in LBW piglets fed diets supplemented with a higher dose of Trp.

To our knowledge, this is the first report on the effect of dietary Trp on glucose metabolism in LBW neonates. The plasma glucose concentration was decreased at 60 min after a meal test in LBW-T0.8. Similarly, others showed that dietary supplementation of Trp reduced hyperglycemia [26] and its injection caused hypoglycemia [62] in rats. Likewise, an intragastric infusion of Trp decreased blood glucose in humans [63], pigs [64], and rats [22]. In contrast, others showed that either the oral administration of Trp in humans [65] or the intragastric administration of Trp in rats [66] increased plasma glucose. Differences in the doses of Trp used, the route of administration (e.g., oral, diet, gastric, and injection), and the experimental units may all lead to discrepancies seen among studies. Intragastric and intravenous tryptophan administration has been reported to produce

a differential effect on plasma glucose and hormones controlling blood glucose, such as glucagon and gastric inhibitory polypeptide (GIP) [66]. It appears that the oral or intragastric administration of Trp at supraphysiological levels stimulates glucagon secretion and hyperglycemia [65,66]. Unlike the abovementioned studies, our data suggest a hypoglycemic role for Trp, which might be due to the disposal of glucose by tryptophan by increasing insulin and incretins [67] and glucose-mediated GIP secretion [64], and the physiological effect of Trp in inhibiting the absorption of glucose from the small intestine [26] and slowing gastric emptying [63,68].

Little is understood about the effect of Trp on biochemical pathways involved in glucose metabolism in LBW neonates. To our knowledge, the intestinal absorption of glucose and the dynamics of glucose metabolism in liver, particularly glycolysis and gluconeogenesis, have not been elucidated in LBW neonates receiving Trp-enriched diets. For the first time, here we show an increased hepatic mRNA abundance of GCK, decreased PEPCK transcript in the liver, and reduced hepatic GLUT2 gene and protein abundance in the LBW-T0.8 group. Further, the protein expression of SGLT1 in the jejunum and GLUT2 in the liver was decreased in the LBW-T0.4 group. Unlike our study, where we showed an increased mRNA abundance of GCK in LBW-T0.8, others have either failed to detect a change in the hepatic GCK mRNA expression in rats fed with diets supplemented with Trp [22] or showed a downregulation in the GCK expression in the liver with a higher dose of dietary Trp in blunt snout bream [69]. Differences in the amount of supplemented Trp and animal species used may contribute to the discrepancy in the findings. GCK is one of the key limiting enzymes in glycolysis in the liver, which converts glucose to glucose-6-phosphate, and its expression is regulated by insulin [70]. The plasma insulin did not change across groups in our study; therefore, the increased expression of GCK in response to a high dose of supplemental Trp suggests the existence of an alternative pathway. GCK has three Trp residues in its structure [71], which may explain the link between the availability of Trp and GCK synthesis. The increased transcript of GCK in the current study is suggestive of an increased glycolysis in

LBW pigs fed with diets supplemented with 0.8% Trp, which may contribute to the reduced hyperglycemia in this group. In line with our findings, others reported a reduced activity for PEPCK following Trp administration in normal and adrenalectomized rats [72]. However, there are other reports showing an increased PEPCK activity following the administration of Trp in rats [73] and mice [74]. In our study, we did not measure the activity of PEPCK; however, the energy balance status of the animals used (e.g., fed vs. fasted) or possible differences in the regulation of gluconeogenesis by nutrients and amino acids, such as Trp, among different animal species may contribute to the controversial effect of Trp on the PEPCK activity in the abovementioned studies. PEPCK is the first key rate-limiting enzyme in hepatic gluconeogenesis [75]. The lower abundance of PEPCK mRNA in LBW-T0.8 in the current study is suggestive of a reduced gluconeogenesis, which may explain the lower plasma glucose concentration in this group. In the present study, we showed a reduced hepatic GLUT2 expression in both the LBW-T0.4 and LBW-T0.8 groups. To our knowledge, there is no published data on the dynamics of hepatic GLUT2 following Trp administration. The major role of GLUT2 in the liver is glucose efflux following gluconeogenesis, rather than glucose uptake [76]. Therefore, the reduced expression of hepatic GLUT2 in LBW-T0.4 and LBW-T0.8 is indicative of a reduced glucose efflux to the bloodstream through hepatocytes. This may explain the lower blood glucose concentration seen in LBW-T0.8 piglets. Further, we report here a reduced protein abundance of SGLT1 in the jejunum of LBW-T0.4 piglets. Previously, L-Trp was shown to have a strong interaction with SGLT1 [26]. Given the important role of SGLT1 in the absorption of glucose in the gut, our data suggest that Trp inhibits the absorption of glucose through the downregulation of intestinal SGLT1. Overall, the reduced blood glucose in LBW-T0.8 might be explained by the increased glycolysis, decreased gluconeogenesis, and reduced hepatic glucose efflux in the LBW-T0.8 group. While the expression of SGLT1 in the jejunum and GLUT2 in the liver was decreased in the LBW-T0.4 group, the blood glucose did not change in the same group. Unlike the higher dose of Trp (T0.8), which changed the expression glycolytic and gluconeogenic enzymes, the lower dose of supplemental Trp appears to only influence the uptake

of glucose from the intestine and efflux of glucose in hepatocytes, which does not seem to be sufficient to change the blood glucose level.

In the current study, for the first time, we show a decreased plasma triglyceride concentration in LBW-T0.4 and LBW-T0.8 piglets. In agreement with our data, dietary Trp supplementation reduced the serum lipids in rats [23] and the hepatic lipids in laying hens and broilers [24,25]. The data on the cellular lipid metabolism in LBW neonates fed with diets supplemented with higher amounts of Trp are scarce. In particular, there is a paucity of data on lipogenesis and lipolysis in LBW neonates fed with diets enriched in Trp. Here, we report a reduced hepatic mRNA abundance of ACC in both LBW-T0.4 and LBW-T0.8 and increased hepatic mRNA abundance of HADH in LBW-T0.4 piglets. ACC is a key enzyme involved in the biosynthesis of lipids, while HADH catalyzes the oxidation of fatty acids during beta oxidation. The downregulation of ACC and upregulation of HADH in the liver suggest that Trp supplementation reduces the lipogenesis but increases the lipolysis in the liver of LBW neonates, which may explain the reduced blood triglycerides. In support of our results, others showed that Trp increased the activity of CPT1 and oxidation of fatty acids [77]. They reported that Trp is involved in lipid metabolism through, serving as a precursor in the synthesis of melatonin [78,79]. Further research is warranted to better understand the role of endocrine signals that trigger alterations in lipid and glucose metabolism in LBW neonates receiving Trp-enriched diets. Given the role of Trp and its metabolism as one of the key modulators of gut microbiota [80] and also the importance of microbiota in glucose and lipid metabolism [81,82], it remains to be determined whether the gut microbiome is involved in alterations in glucose and lipid metabolism following Trp supplementation. Further, there appears to be a minor link between Trp metabolism metabolites and iron metabolism biomarkers [83]. Indicators of tryptophan metabolism have been shown to be positively correlated with hemoglobin and markers of iron metabolism [83]. Whether Trp-induced changes in iron metabolism play a role in the metabolic outcome of Trp-enriched diets requires further investigation.

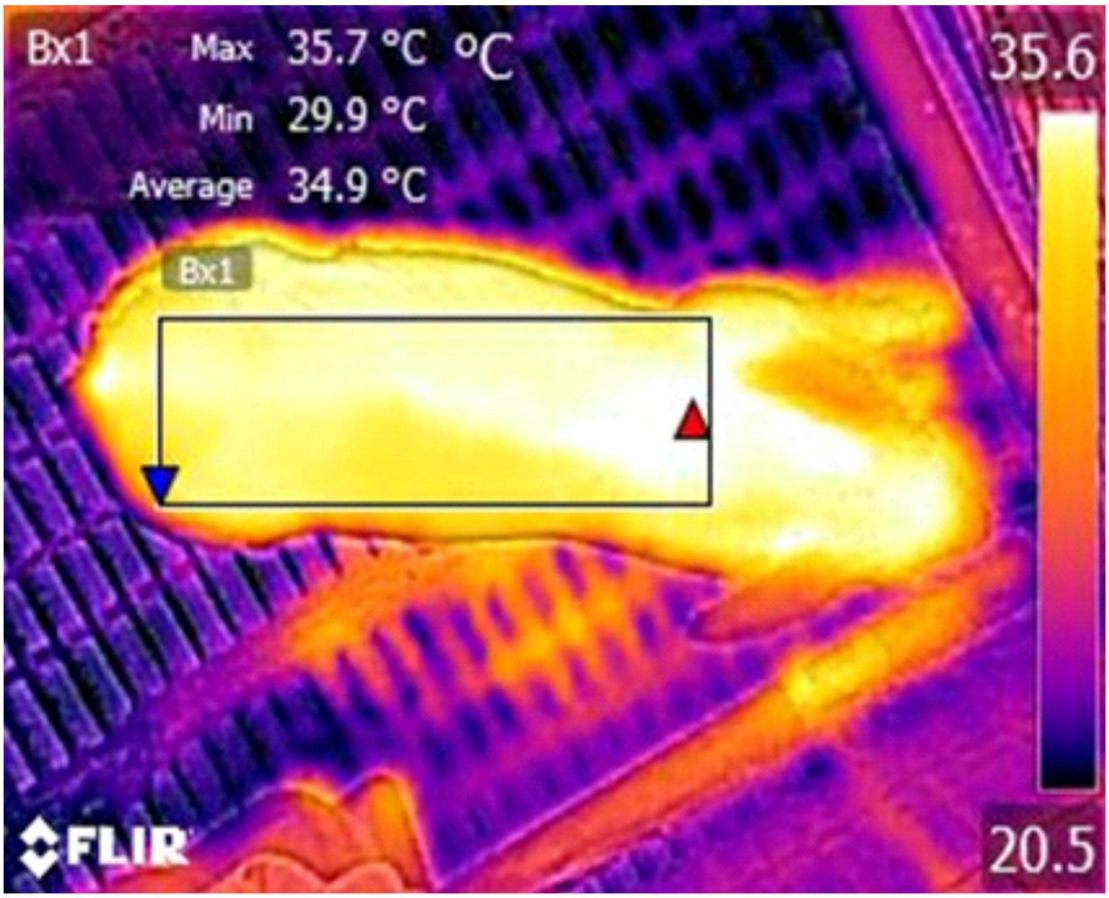
While the role of brain serotonin, as an anorexigenic hormone in the regulation of feeding behavior, is documented [27,28,29,30,84], little is known of the role of peripheral serotonin in appetite regulation. In the present study, blocking 5-HT3 receptors with ondansetron reduced the feed intake in all groups. This was in line with a previous report, where ondansetron reduced the feed intake in rats [85]. Further investigation is needed to determine whether 5-HT3 receptors and peripheral serotonin are involved in the regulation of ingestive behavior in LBW piglets. More specifically, ondansetron decreased the intake transiently in LBW-T0, but that reduced the feed intake throughout the day in the LBW-T0.8 and NBW-T0 groups. This is indicative of a higher serotonergic signaling in the LBW-T0.8 and NBW-T0 groups, but a lower serotonergic tone in LBW-T0 piglets. The gastrointestinal tract is the main site for the conversion of Trp into serotonin [27,30,86], and Trp availability has been shown to enhance serotonin synthesis through the hydroxylation of Trp in a rate-limiting step by the action of tryptophan hydroxylase [87]. Therefore, our data suggest a higher availability of Trp in the LBW-T0.8 and NBW-T0 groups for the synthesis of gut-derived serotonin. In support of our data, a lower serotonin concentration in LBW piglets has been reported [27]. Since >95% of serotonin is produced in the gastrointestinal tract [27,30,86], the lower blood serotonin is suggestive of a reduced serotonin synthesis in the intestinal enterochromaffin cells of LBW piglets. Since the synthesized serotonin in enterochromaffin cells is stored in platelets and released after stimulation [88], further research is required to understand whether a lower peripheral serotonin is related with a higher rate of thrombocytopenia in LBW neonates [89].

3.6. Conclusions

To our knowledge, this is the first study assessing the effect of dietary Trp on glucose and lipid metabolism in LBW neonates. We demonstrated that supplemental Trp improves the glucose and lipid metabolism in an LBW piglet model through reducing hepatic lipogenesis, gluconeogenesis, and glucose efflux and increasing lipolysis and glycolysis. Further, we provided evidence of the

role of peripheral serotonin in the regulation of feeding behavior and a lower serotonergic signaling in LBW piglets receiving no Trp supplement. Further research on the endocrine regulation of metabolic pathways involved in glucose and lipid metabolism following the administration of Trp in LBW neonates is required.

3.7. Appendices



Appendix Figure 3A.1. A representative screenshot of a thermal image.

Dorsal surface body mean temperature was obtained by drawing a rectangular in the entire back of piglets approximately from shoulders to the rump of the animal using a rectangular drawing tool of FLIR camera software (FLIR Research Studio, Wilsonville, OR)

Appendix Table 3A.1. Analyzed chemical composition of diets (As-fed basis)

Ingredients %	**Diets[1]**		
	T0	**T0.4**	**T0.8**
Whey powder[2]	52.92	52.92	52.92
Whey protein concentrate 36.17%[2]	24.58	24.58	24.58
Corn oil[2]	12.00	12.00	12.00
Sodium caseinate[2]	5.01	5.01	5.01
Dextrose[2]	1.02	0.97	0.91
Lactose[2]	1.00	1.00	1.00
Dicalcium phosphate 18.5%[2]	1.00	1.00	1.00
L-Alanin[2]	0.87	0.52	0.18
L-Tryptophan[2]	0.00	0.40	0.80
Limestone[2]	0.43	0.43	0.43
L-Lysine sulphate[2]	0.07	0.07	0.07
DL-Methionine[2]	0.12	0.12	0.12
L-Arginine[2]	0.21	0.21	0.21
L-Phenylalanine[2]	0.29	0.29	0.29
L-Histidine[2]	0.11	0.11	0.11
Vitamin premix[3]	0.25	0.25	0.25
Mineral premix[4]	0.12	0.12	0.12
Calculated Chemical Composition[5]			
Dry matter, %	95.64	95.64	95.65
ME, Mcal/kg	4.37	4.37	4.37
Crude protein, %	22.60	22.60	22.60
Crude fat, %	13.50	13.50	13.50
Lactose, %	51.34	51.34	51.34
SID Lysine, %	1.57	1.57	1.57
SID Threonine, %	1.00	1.00	1.00
SID Methionine, %	0.48	0.48	0.48
SID Tryptophan, %	0.31	0.71	1.11
SID Isoleucine, %	1.08	1.08	1.08
SID Valine, %	1.05	1.05	1.05
SID Arginine, %	0.65	0.65	0.65
SID Histidine, %	0.50	0.50	0.50
SID Lucine, %	1.83	1.83	1.83
SID Phenylalanine, %	0.94	0.94	0.94
SID Phe + Tyrosine, %	1.48	1.48	1.48
Calcium, %	0.90	0.90	0.90
Total phosphorus, %	0.73	0.73	0.73
Potassium, %	1.45	1.45	1.45

[1]**T0**: basal diet without supplemented L-tryptophan (Trp); T0.4: basal diet supplemented with 0.4% Trp; T0.8: basal diet supplemented with 0.8% Trp.[2]Whey powder, whey protein concentrate, corn oil, dextrose, lactose, dicalcium phosphate, limestone and L-arginine were obtained from Nutra Blend, LLC (Neosho, MO). Sodium caseinate was obtained from AMCO PROTEIN (Burlington, NJ). DL-methionine (99%) (MetAMINO®) and L-lysine (Biolys®) were obtained from Evonik (Kennesaw, GA). L-tryptophan (98%) was purchased from Ajinomoto (Overland Park, KS). L-valine (96.5%), L-histidine, L-phenylalanine and L-alanine was obtained from Ajinomoto Health & Nutrition North America, Inc. (Raleigh, NC). [3]Vitamin premix were purchased from Nutra Blend, LLC (Neosho, MO). Vitamin premix (per kg) contained: vitamin A, 1,650,000 IU; vitamin D_3, 660,000 IU; vitamin E, 17,600 IU; vitamin K, 1,320 mg; vitamin B12, 13.2 mg; niacin, 19,800 mg; pantothenic acid, 11,000 mg; riboflavin, 3,300 mg; phytase, 299,376 FYT. [4]Mineral premix were purchased from Nutra Blend, LLC (Neosho, MO). Mineral premix contained: copper, 11,000 ppm; iodine, 198 ppm; iron, 73,000 ppm; manganese, 22,000 ppm; selenium, 198 ppm; zinc, 73,000. [5]National Swine Nutrition Guide (Version 2.1 Metric, ©2012 U.S. Pork Center of Excellence)

Appendix Table 3A.2. Chemical composition of supplemental amino acids and whey powder used in diets (As-fed basis)

Chemical Composition	**Ingredients**[1]			
	Arg	His	Phe	Whey powder
Dry matter, %	99.6	99.5	99.6	94.4
Crude protein, %	202.1	170.7	52.9	13.2
Crude fiber, %	<1.0	<1.0	<1.0	<1.0
Calcium, %	0.02	0.01	0.07	0.60
Phosphorus, %	0.01	0.01	<0.01	0.62

[1]The "less than" symbol (<) indicates the result was lower than the reporting limit for this laboratory ServiTech Laboratories (Dodge City, KS).

Appendix Table 3A.3. Amino acid profile and crude protein of whey protein concentrate used in diets (As-fed basis)

Items	Whey Protein Concentrate
Taurine*,%	0.15
Aspartic acid, %	3.82
Threonine, %	2.45
Serine, %	1.44
Glutamic acid, %	6.23
Proline, %	2.13
Glycine, %	0.68
Alanine, %	1.89
Cysteine, %	0.86
Valine, %	2.20
Methionine, %	0.67
Isoleucine, %	2.46
Leucine, %	3.89
Tyrosine, %	0.89
Phenylalanine, %	1.21
Hydroxylysine, %	0.02
Ornithine*, %	0.06
Lysine, %	3.33
Histidine, %	0.68
Arginine, %	0.92
Tryptophan, %	0.74
Crude protein#, %	36.17

* Non-proteinogenic amino acids

Crude protein= %N×6.25

Appendix Table 3A.4. Analyzed chemical composition of diets (As-fed basis)

Items	Diets[1]		
	T0	**T0.4**	**T0.8**
Taurine[2],%	0.13	0.12	0.12
Aspartic acid,%	1.84	1.88	1.87
Threonine,%	1.16	1.19	1.18
Serine,%	0.88	0.92	0.90
Glutamic acid,%	3.36	3.43	3.38
Proline,%	1.33	1.35	1.32
Lanthionine[2]	0.12	0.11	0.15
Glycine,%	0.39	0.40	0.40
Alanine,%	1.46	1.30	1.09
Cysteine,%	0.38	0.37	0.37
Valine,%	1.20	1.22	1.21
Methionine,%	0.45	0.56	0.47
Isoleucine,%	1.16	1.18	1.17
Leucine,%	1.98	2.03	2.01
Tyrosine,%	0.64	0.67	0.64
Phenylalanine,%	0.80	0.95	0.97
Hydroxylysine,%	0.04	0.04	0.02
Ornithine[2],%	0.01	0.01	0.01
Lysine,%	1.76	1.71	1.70
Histidine,%	0.49	0.51	0.52
Arginine,%	0.80	0.84	0.61
Tryptophan,%	0.36	0.84	1.29
Dry matter, %	95.3	95.8	95.0
Crude protein[3],%	21.5	21.4	20.7
Crude fiber, %	<1.0	<1.0	<1.0
Calcium, %	0.96	0.91	0.97
Phosphorus, %	0.72	0.67	0.71

Appendix Table 3A.5. The sequences [forward (F) and reveres (R)], location on template, amplicon size (bp), and GenBank accession numbers for primers used for reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR)

Genes[1]	Sequence (5' → 3')	Location on template	Amplicon length (bp)	GenBank accession no.
FAS	**F:** CTGCTGAAGCCTAACTCCTCG **R:** TTGCTCCTTGGAACCGTCTG	584 - 604 771 - 790	207	NM_001099930.1
ACC	**F:** ATGTTTCGGCAGTCCCTGAT **R:** TGTGGACCAGCTGACCTTGA	4870 - 4889 4983 - 5002	133	NM_001114269.1
HSL	**F:** GCTCCCATCGTCAAGAATC **R:** TAAAGCGAATGCGGTCC	2043 - 2061 2291 - 2307	265	NM_214315.3
PPARα	**F:** CATCCTCGCGGGAAAGG **R:**GGCCATACACAGTGTCTCCATGT	722 - 738 769 - 791	70	NM_001044526.1
SREBP-1	F: CGGACGGCTCACAATGC R: GACGGCGGATTTATTCAGCTT	986 - 1002 1079 - 1099	114	NM_214157.1
HADH	**F:** GCCATCGTGGAGAACCTGAA **R:** GAAATGGAGCCCGGCAAATC	461 - 480 600 - 619	159	NM_214331.1
PGC1α	**F:** GATGTGTCGCCTTCTTGTTC **R:** CATCCTTTGGGGTCTTTGAG	1629 - 1648 1702 - 1721	93	NM_213963.2
LPL	**F:** CCCTATACAAGAGGGAACCGGAT **R:** CCGCCATCCAGTCGATAAACGT	448 - 470 564 - 580	138	NM_214286.1
CD36	**F:** CTGGTGCTGTCATTGGAGCAGT **R:** CTGTCTGTAAACTTCCGTGCCTGTT	443 - 464 579 - 603	161	NM_001044622.1
GCK	**F:** CCGACTTCCTGGACAAGCAT **R:** ATCGTGGCCACAGTGTCATT	1040 -1059 1258 -1277	238	XM_003134883.2
PFKL	**F:** ACTCCCTTCGACCGGAACTA **R:** TGCTCAAAGTCGGTGTCCTC	2119 - 2138 2296 - 2315	197	XM_021071510.1
GLUT2	**F:** GGTTCATGGTGGCCGAGTT **R:** ATTGCGGGTCCAGTTGC	1260 - 1278 1326 - 1342	83	NM_001097417.1
PEPCK	**F:** CTGGGAAGGCATTGATCAGC **R:** AGCGAGAGTTAGGATGTACA	1336 - 1335 1426 - 1445	110	NM_001161753.1
G6PC	**F:** TGAACGTCTGTCTGTCACGA **R:** ATACTTCTTGAGGCTGGCGT	491 - 510 608 - 627	137	NM_001113445.1
GLUT1	**F:** GGAGATGAAGGAGGAGAGCC **R:** TAGAAAACCGCGTTGATGCC	982 - 1001 1112 - 1131	150	XM_021096908.1
PC	**F:** GGACTTCACTGCCACCTTTG **R:** GCTCCACCTCAAACTCCTCT	3063 - 3082 3135 - 3154	92	NM_214349.1
PKLR	**F:** CCCACTGAAGTCACCGCTAT **R:** GAGGAAGCCACGGAGTTTT	1357 – 1376 1626 - 1644	288	XM_021089721.1
β-Actin	**F:** CTGCGGCATCCACGAAACT **R:** AGGGCCGTGATCTCCTTCTG	944 - 962 1071 - 1090	147	XM_003124280.5

[1]FAS = fatty acid synthase; ACC = acetyl-CoA carboxylase; HSL = Hormone-sensitive lipase; PPARα = Peroxisome proliferator activated receptor alpha; SREBP-1 = sterol regulatory element binding transcription factor 1; HADH = Hydroxyacyl-CoA dehydrogenase; PGC1α = PPARG coactivator 1 alpha; LPL = lipoprotein lipase; CD36 = cluster of differentiation 36 molecule, GCK = glucokinase; PFKL = phosphofructokinase, liver type; GLUT2 = glucose transporter 2; PEPCK = phosphoenolpyruvate carboxykinase; G6PC = glucose-6-phosphatase catalytic; GLUT1 = glucose transporter 1; PC = pyruvate carboxylase; PKLR = pyruvate kinase, liver and RBC .

Appendix Table 3A.6. The host, dilution and supplier of primary and secondary antibodies for immunoblotting

Antibodies	Host	Dilution	Vendor
Anti-Tryptophan hydroxylase 2 (TPH2)	Goat	1:500	Abcam, Cambridge, MA, #ab121013
Anti-Sodium-glucose co-transporter 1 (SGLT-1)	Rabbit	1:2000	Thermo Scientific, Rockford, IL, #PA5-28240
Anti-Carnitine palmitoyltransferase I α (CPT1α)	Rabbit	1:3000	Thermo Scientific, Rockford, IL, #15184-1-AP
Anti-Glucose transporter 2 (GLUT-2)	Rabbit	1:500	Thermo Scientific, Rockford, IL, #720238
Anti-GAPDH [6C5] (HRP)	Mouse	1:5000	Abcam, Cambridge, MA, #ab105428
Anti-Goat IgG H&L (HRP)	Donkey	1:10000	Abcam, Cambridge, MA, #ab205723
Anti-Rabbit IgG H&L (HRP)	Goat	1:1600	Abcam, Cambridge, MA, #ab205718

3.8. References

1. Ananth, C.V.; Vintzileos, A.M. Distinguishing pathological from constitutional small for gestational age births in population-based studies. Early Hum. Dev. 2009, 85, 653–658, doi:10.1016/j.earlhumdev.2009.09.004.

2. Bernstein, I.; Gabbe, S.G. Intrauterine growth restriction. In Obstetrics: Normal and Problem Pregnancies, 3rd ed.; Gabbe, S.G., Niebyl, J.R., Simpson, J.L., Annas, G.J., et al., Eds.; Churchill Livingstone: New York, NY, USA, 1996; Volume 3, pp. 863–886.

3. Creasy, R.K.; Resnik, R. Intrauterine growth restriction. In Maternal-Fetal Medicine: Principles and Practice, 3rd ed.; Creasy, R.K., Resnik, R., Eds.; Saunders: Philadelphia, PA, USA, 1994; pp. 558–574.

4. Martin, J.A.; Hamilton, B.E.; Osterman, M.J.K.; Driscoll, A.K. Births: Final Data for 2019. National Vital Statistics Reports; National Center for Health Statistics: Hyattsville, MD, USA, 2021; Volume 70, doi:10.15620/cdc:100472.

5. Ewing, A.C.; Ellington, S.R.; Shapiro-Mendoza, C.K.; Barfield, W.D.; Kourtis, A.P. Full-term small-for-gestational-age newborns in the US: Characteristics, trends, and morbidity. Matern. Child Health J. 2017, 21, 786–796, doi:10.1007/s10995-016-2165-z.

6. Fontaine, M.A.; Diane, A.; Singh, V.P.; Mangat, R.; Krysa, J.A.; Nelson, R.; Willing, B.P.; Proctor, S.D. Low birth weight causes insulin resistance and aberrant intestinal lipid metabolism independent of microbiota abundance in Landrace–Large White pigs. FASEB J. 2019, 33, 9250–9262, doi:10.1096/fj.201801302RR.

7. Giapros, V.; Vavva, E.; Siomou, E.; Kolios, G.; Tsabouri, S.; Cholevas, V.; Bairaktari, E.; Tzoufi, M.; Challa, A. Low-birth-weight, but not catch-up growth, correlates with insulin resistance and resistin level in SGA infants at 12 months. J. Matern. Fetal. Neonatal. Med. 2017, 30, 1771–1776, doi:10.1080/14767058.2016.1224838.

8. Li, N.; Wang, W.; Wu, G.; Wang, J. Nutritional support for low birth weight infants: Insights from animal studies. Br. J. Nutr. 2017, 117, 1390–1402, doi:10.1017/S000711451700126X.

9. Wang, X.; Cui, Y.; Tong, X.; Ye, H.; Li, S. Glucose and lipid metabolism in small-for-gestational-age infants at 72 hours of age. J. Clin. Endocrinol. Metab. 2007, 92, 681–684, doi:10.1210/jc.2006-1281.

10. Xu, Y.; Chen, S.; Yang, H.; Gong, F.; Wang, L.; Jiang, Y.; Yan, C.; Zhu, H.; Pan, H. Decreased insulin sensitivity and abnormal glucose metabolism start in preadolescence in low-birth-weight children—Meta-analysis and systematic review. Prim. Care Diabetes. 2019, 13, 391–398, doi:10.1016/j.pcd.2019.03.012.

11. Lane, R.H.; Flozak, A.S.; Ogata, E.S.; Bell, G.I.; Simmons, R.A. Altered hepatic gene expression of enzymes involved in energy metabolism in the growth-retarded fetal rat. Pediatr. Res. 1996, 39, 390–394, doi:10.1203/00006450-199603000-00003.

12. Peterside, I.E.; Selak, M.A.; Simmons, R.A. Impaired oxidative phosphorylation in hepatic mitochondria in growth-retarded rats. Am. J. Physiol. Endocrinol. Metab. 2003, 285, 1258–1266, doi:10.1152/ajpendo.00437.2002.

13. Gomo, Z.A.R.; Nyatanga, K.; Chifamba, J.; Chinyanga, H.M.; Taderera, T.; Gwaunza, L.T.; Mushayamano, T.; Mahachi, C. Glucose tolerance study in low and normal birth weight young adults. Cent. Afr. J. Med. 2013, 59, 38–42.

14. Ying, Z.; Ge, X.; Zhang, H.; Su, W.; Li, Y.; Zhou, L.; Zhang, L.; Wang, T. Effects of dietary methionine restriction on postnatal growth, insulin sensitivity, and glucose metabolism in intrauterine growth retardation pigs at 49 and 105 d of age. J. Anim. Sci. 2019, 97, 610–619, doi:10.1093/jas/sky457.

15. Ying, Z.; Zhang, H.; Su, W.; Zhou, L.; Wang, F.; Li, Y.; Zhang, L.; Wang, T. Dietary methionine restriction alleviates hyperglycemia in pigs with intrauterine growth restriction by enhancing hepatic protein kinase B signaling and glycogen synthesis. J. Nutr. 2017, 147, 1892–1899, doi:10.3945/jn.117.253427.

16. Jones, J.N.; Gercel-Taylor, C.; Taylor, D.D. Altered cord serum lipid levels associated with small for gestational age infants. Obstet. Gynecol. 1999, 93, 527–531, doi:10.1016/S0029-7844(98)00489-X.

17. Liu, C.; Lin, G.; Wang, X.; Wang, T.; Wu, G.; Li, D.; Wang, J. Intrauterine growth restriction alters the hepatic proteome in fetal pigs. J. Nutr. Biochem. 2013, 24, 954–959, doi:10.1016/j.jnutbio.2012.06.016.

18. Cooper, J.E. The use of the pig as an animal model to study problems associated with low birthweight. Lab. Anim. 1975, 9, 329–336, doi:10.1258/002367775780957188.

19. Ferenc, K.; Pietrzak, P.; Godlewski, M.M.; Piwowarski, J.; Kilianczyk, R.; Guilloteau, P.; Zabielski, R. Intrauterine growth retarded piglet as a model for humans–studies on the perinatal development of the gut structure and function. Reprod. Biol. 2014, 14, 51–60, doi:10.1016/j.repbio.2014.01.005.

20. Eiby, Y.A.; Wright, L.L.; Kalanjati, V.P.; Miller, S.M.; Bjorkman, S.T; Keates, H.L.; Lumbers, E.R.; Colditz, P.B.; Lingwood, B.E. A pig model of the preterm neonate: Anthropometric and physiological characteristics. PLoS ONE 2013, 8, e68763, doi:10.1371/journal.pone.0068763.

21. Odle, J.; Lin, X.; Jacobi, S.K.; Kim, S.W; Stahl, C.H. The suckling piglet as an agrimedical model for the study of pediatric nutrition and metabolism. Annu. Rev. Anim. Biosci. 2014, 2, 419–444, doi:10.1146/annurev-animal-022513-114158.

22. Ardiansyah, S.; Shirakawa, H.; Inagawa, Y.; Koseki, T.; Komai, M. Regulation of blood pressure and glucose metabolism induced by L-tryptophan in stroke-prone spontaneously hypertensive rats. Nutr. Metab. 2011, 8, 1–7, doi:10.1186/1743-7075-8-45.

23. Ruan, Z.; Yang, Y.; Wen, Y.; Zhou, Y.; Fu, X.; Ding, S.; Liu, G.; Yao, K.; Wu, X.; Deng, Z.; Wu, G.; Yin, Y. Metabolomic analysis of amino acid and fat metabolism in rats with l-tryptophan supplementation. Amino Acids 2014, 46, 2681–2691, doi:10.1007/s00726-014-1823-y.

24. Akiba, Y.; Takahashi, K.; Horiguchi, M.; Ohtani, H.; Saitoh, S.; Ohkawara, H. L-tryptophan alleviates fatty liver and modifies hepatic microsomal mixed function oxidase in laying hens. Comp. Biochem. Physiol. A Physiol. 1992, 102, 769–774, doi:10.1016/0300-9629(92)90738-c.

25. Rogers, S.R.; Pesti, G.M. The influence of dietary tryptophan on broiler chick growth and lipid metabolism as mediated by dietary protein levels. Poult. Sci. 1990, 69, 746–756, doi:10.3382/ps.0690746.

26. Inubushi, T.; Kamemura, N.; Oda, M.; Sakurai, J.; Nakaya, Y.; Harada, N.; Suenaga, M.; Matsunaga, Y.; Ishidoh, K.; Katunuma, N. L-tryptophan suppresses rise in blood glucose and preserves insulin secretion in type-2 diabetes mellitus rats. J. Nutr. Sci. Vitaminol. 2012, 58, 415–422, doi:10.3177/jnsv.58.415.

27. Willemen, S.A.; Che, L.; Dewilde, S.; Van Hauwaert, M.L.; De Vos, M.; Huygelen, V.; Fransen, E.; Tambuyzer, B.R.; Casteleyn, C.; Van Cruchten, S.; Van Ginneken, C. Enteric and serological distribution of serotonin and its precursor tryptophan in perinatal low and normal weight piglets. Animals 2014, 8, 792–799, doi:10.1017/S1751731114000317.

28. Cui, R.J.; Roberts, B.L.; Zhao, H.; Zhu, M.; Appleyard, S.M. Serotonin activates catecholamine neurons in the solitary tract nucleus by increasing spontaneous glutamate inputs. J. Neurosci. 2012, 32, 16530–16538, doi:10.1523/JNEUROSCI.1372-12.

29. Fetissov, S.O.; Meguid, M.M.; Chen, C.; Miyata, G. Synchronized release of dopamine and serotonin in the medial and lateral hypothalamus of rats. Neuroscience 2000, 101, 657–663, doi:10.1523/JNEUROSCI.1372-12.2012.

30. Lam, D.D.; Garfield, A.S.; Marston, O.J.; Shaw, J.; Heisler, L.K. Brain serotonin system in the coordination of food intake and body weight. Pharmacol. Biochem. Behav. 2010, 97, 84–91, doi:10.1016/j.pbb.2010.09.003.

31. Dépôt, M.; Caillé, G.; Mukherjee, J.; Katzman, M.A.; Cadieux, A.; Bradwejn, J. Acute and chronic role of 5-HT3 neuronal system on behavioral and neuroendocrine changes induced by intravenous cholecystokinin tetrapeptide administration in humans. Neuropsychopharmacology 1999, 20, 177–187, doi:10.1016/S0893-133X(98)00074-8.

32. McGlone, J. Guide for the Care and Use of Agricultural Animals in Research and Teaching, 3rd ed. Available online: https://www.aaalac.org/about/ag_guide_3rd_ed.pdf (accessed on 4 March 2019).

33. Rafiee-Tari, N.; Fan, M.Z.; Archbold, T.; Arranz, E.; Corredig, M. Effect of milk protein composition and amount of β-casein on growth performance, gut hormones, and inflammatory cytokines in an in vivo piglet model. J. Dairy Sci. 2019, 102, 8604–8613, doi:10.3168/jds.2018-15786.

34. Wang, Y.; Zhang, L.; Zhou, G.; Liao, Z.; Ahmad, H.; Liu, W.; Wang, T. Dietary L-arginine supplementation improves the intestinal development through increasing mucosal Akt and mammalian target of rapamycin signals in intra-uterine growth retarded piglets. Br. J. Nutr. 2012, 108, 1371–1381, doi:10.1017/S0007114511006763.

35. Zheng, P.; Song, Y.; Tian,Y.; Zhang, H.; Yu, B.; He, J.; Mao, X.; Yu, J.; Luo, Y.; Luo, J.; Huang, Z.; Tian, G.; Chen, H.; Chen, D. Dietary arginine supplementation affects intestinal function by enhancing antioxidant capacity of a nitric oxide–independent pathway in low-birth-weight piglets. J. Nutr. 2018, 148, 1751–1759, doi:10.1093/jn/nxy198.

36. DeRouchey, J.M.; Goodband, R.D.; Tokach, M.D.; Nelssen, J.L.; Dritz, S.S. Nursery Swine Nutrient Recommendations and Feeding Management. In National Swine Nutrition Guide; Meisinger, D.J. Ed.; U.S. Pork Center of Excellence: Ames, IA, USA, 2010; pp. 65–79.

37. Ebert, A.R.; Berman, A,S.; Harrell, R.J.; Kessler, A.M.; Cornelius, S.G.; Odle, J. Vegetable proteins enhance the growth of milk-fed piglets, despite lower apparent ileal digestibility. J. Nutr. 2005, 135, 2137–2143, doi:10.1093/jn/135.9.2137.

38. Habibi, M.; Shili, C.; Sutton, J.; Goodarzi, P.; Maylem, E.R;. Spicer, L.; Pezeshki, A. Branched-chain amino acids partially recover the reduced growth of pigs fed with protein-restricted diets through both central and peripheral factors. Anim. Nutr. 2021, doi:10.1016/j.aninu.2021.02.002.

39. Kim, S.W.; McPherson, R.L.; Wu, G. Dietary arginine supplementation enhances the growth of milk-fed young pigs. J. Nutr. 2004, 134, 625–630, doi:10.1093/jn/134.3.625.

40. Madsen, J.G.; Mueller, S.; Kreuzer, M.; Bigler, M.B.; Silacci, P.; Bee, G. Milk replacers supplemented with either L-arginine or L-carnitine potentially improve muscle maturation of early reared low birth weight piglets from hyperprolific sows. Animals 2018, 12, 43–53, doi:10.1017/S175173111700132X.

41. Jensen, G.M.; Grøndahl, M.L.; Nielsen, C.G.; Skadhauge, E.; Olsen, J.E.; Hansen, M.B. Effect of ondansetron on Salmonella typhimurium-induced net fluid accumulation in the pig jejunum in vivo. Comp. Biochem. Physiol. A Physiol. 1997, 118, 297–299, doi:10.1016/s0300-9629(96)00308-8.

42. Spring, S.; Premathilake, H.; Bradway, C.; Shili, C.; DeSilva, U.; Carter, S.; Pezeshki, A. Effect of very low-protein diets supplemented with branched-chain amino acids on energy balance,

plasma metabolomics and fecal microbiome of pigs. Sci. Rep. 2020, 10,1–16, doi:10.1038/s41598-020-72816-8.

43. Spring, S.; Premathilake, H.; DeSilva, U.; Shili, C.; Carter, S.; Pezeshki, A. Low protein-high carbohydrate diets alter energy balance, gut microbiota composition and blood metabolomics profile in young pigs. Sci. Rep. 2020, 10, 1–15, doi:10.1038/s41598-020-60150-y.

44. Shili, C.N.; Broomhead, J.N.; Spring, S.C.; Lanahan, M.B.; Pezeshki, A. A novel corn-expressed phytase improves daily weight gain, protein efficiency ratio and nutrients digestibility and alters fecal microbiota in pigs fed with very low protein diets. Animals 2020, 10, 1926, doi:10.3390/ani10101926.

45. AOAC. Official Method 982.30 E (a, b, c), chp, 45.3. 05 2006. Amino Acid profile without Tryptophan AAP-T; Association of Official Analytical Chemists: Gaithersburg, USA, 2006.

46. Bakken, G.S. A heat transfer analysis of animals: Unifying concepts and the application of metabolism chamber data to field ecology. J. Theor. Biol. 1976, 60, 337–384, doi:10.1016/0022-5193(76)90063-1.

47. Ferreira, V.M.; Francisco, N.S.; Belloni, M.; Aguirre, G.M.; Caldara, F.R.; Nääs, I.A.; Garcia, R.G.; Almeida Paz, I.C.; Polycarpo, G.V. Infrared thermography applied to the evaluation of metabolic heat loss of chicks fed with different energy densities. Braz. J. Poult. Sci. 2011, 13, 113–118, doi:10.1590/S1516-635X2011000200005.

48. Digalakis, M.; Papamichail, M.; Glava, C.; Grammatoglou, X.; Sergentanis, T.N.; Papalois, A.; Bramis, J. Interposition of a reversed jejunal segment enhances intestinal adaptation in short bowel syndrome: An experimental study on pigs. J. Surg. Res. 2011, 171, 551–557, doi:10.1016/j.jss.2010.06.035.

49. Pezeshki, A.; Muench, G.P.; Chelikani, P.K. Short communication: Expression of peptide YY, proglucagon, neuropeptide Y receptor Y2, and glucagon-like peptide-1 receptor in bovine peripheral tissues. J. Dairy Sci. 2012, 95, 5089–5094, doi:10.3168/jds.2011-5311.

50. Shili, C.N.; Habibi, M.; Sutton, J.; Barnes, J.; Burch-Konda, J.; Pezeshki, A. Effect of a phytogenic water additive on growth performance, blood metabolites and gene expression of amino acid transporters in nursery pigs fed with low-protein/high-carbohydrate diets. Animals 2021, 11, 555, doi:10.3390/ani11020555.

51. Chen, G.; Zhang, J.; Zhang, Y.; Liao, P.; Li, T.; Chen, L.; Yin, Y.; Wang, J.; Wu, G. Oral MSG administration alters hepatic expression of genes for lipid and nitrogen metabolism in suckling piglets. Amino Acids 2014, 46, 245–250, doi:10.1007/s00726-013-1615-9.

52. Duran-Montgé, P.; Theil, P.K.; Lauridsen, C.; Esteve-Garcia E. Dietary fat source affects metabolism of fatty acids in pigs as evaluated by altered expression of lipogenic genes in liver and adipose tissues. Animal 2009, 3, 535–542, doi:10.1017/S1751731108003686.

53. Li, S.; Wang, H.; Wang, X.; Wang, Y.; Feng, J. Betaine affects muscle lipid metabolism via regulating the fatty acid uptake and oxidation in finishing pig. J. Anim. Sci. Biotechnol. 2017, 8, 1–9, doi:10.1186/s40104-017-0200-6.

54. He, D.; Ma, J.; Long, K.; Wang, X.; Li, X.; Jiang, A.; Li, M. Differential expression of genes related to glucose metabolism in domesticated pigs and wild boar. Biosci. Biotechnol. Biochem. 2017, 81, 1478–1483, doi:10.1080/09168451.2017.1322893.

55. Kang, P.; Liu, Y.; Zhu, H.; Zhang, J.; Shi, H.; Li, S.; Pi, D.; Leng, W.; Wang, X.; Wu, H.; Hou, Y. The effect of dietary asparagine supplementation on energy metabolism in liver of weaning pigs when challenged with lipopolysaccharide. Asian Australas J. Anim. Sci. 2018, 31, 548–555, doi:10.5713/ajas.17.0426.

56. Xie, C.; Wang, Q.; Wang, J.; Tan, B.; Fan, Z.; Deng, Z.Y.; Wu, X.; Yin, Y. Developmental changes in hepatic glucose metabolism in a newborn piglet model: A comparative analysis for suckling period and early weaning period. Biochem. Biophys. Res. Commun. 2016, 470, 824–830, doi:10.1016/j.bbrc.2016.01.114.

57. Yin, J.; Liu, M.; Ren, W.; Duan, J.; Yang, G.; Zhao, Y.; Fang, R.; Chen, L.; Li, T.; Yin, Y. Effects of dietary supplementation with glutamate and aspartate on diquat-induced oxidative stress in piglets. PLoS ONE 2015, 10, e0122893, doi:10.1371/journal.pone.0122893.

58. Zuo, F.; Gu, Q.; Li, S.; Wei, H.; Peng, J. Effects of Different Methionine Sources on Methionine Metabolism in the IPEC-J2 Cells. Biomed. Res. Int. 2019, 1–11, doi:10.1155/2019/5464906.

59. Pezeshki; A.; Chelikani, P.K. Effects of Roux-en-Y gastric bypass and ileal transposition surgeries on glucose and lipid metabolism in skeletal muscle and liver. Surg. Obes. Relat. Dis. 2014, 10, 217–228, doi:10.1016/j.soard.2013.09.017.

60. Pezeshki, A.; Zapata, R.C.; Singh, A.; Yee, N.J.; Chelikani, P.K. Low protein diets produce divergent effects on energy balance. Sci. Rep. 2016, 6, 1–13, doi:10.1038/srep25145.

61. Pezeshki, A.; Fahim, A.; Chelikani, P.K. Dietary whey and casein differentially affect energy balance, gut hormones, glucose metabolism, and taste preference in diet-induced obese rats. J. Nutr. 2015, 145, 2236–2244, doi:10.3945/jn.115.213843.

62. Smith, S.A.; Pogson, C.L. Tryptophan and the control of plasma glucose concentrations in the rat. Biochem. J. 1977, 168, 495–506, doi:10.1042/bj1680495.

63. Ullrich, S.S.; Fitzgerald, P.C.E.; Giesbertz, P.; Steinert, R.E.; Horowitz, M.; Feinle-Bisset, C. Effects of intragastric administration of tryptophan on the blood glucose response to a nutrient drink and energy intake, in lean and obese men. Nutrients 2018, 10, 463, doi:10.3390/nu10040463.

64. Ponter, A.A.; Sève, B.; Morgan, L.M. Intragastric tryptophan reduces glycemia after glucose, possibly via glucose-mediated insulinotropic polypeptide, in early-weaned piglets. J. Nutr. 1994, 124, 259–267, doi:10.1093/jn/124.2.259.

65. Hedo, J.A.; Villanueva, M.L.; Marco, J. Elevation of plasma glucose and glucagon after tryptophan ingestion in man. Metabolism 1977, 26, 1131–1134, doi:10.1016/0026-0495(77)90040-3.

66. Tsiolakis, D.; Marks, V. The differential effect of intragastric and intravenous tryptophan on plasma glucose, insulin, glucagon, GLI and GIP in the fasted rat. Horm. Metab. Res. 1984, 16, 226–229, doi:10.1055/s-2007-1014752.

67. Lin, H.V.; Efanov, A.M.; Fang, X.; Beavers, L.S.; Wang, X.; Wang, J.; Gonzalez Valcarcel, I.C.; Ma, T. GPR142 controls tryptophan-induced insulin and incretin hormone secretion to improve glucose metabolism. PLoS ONE 2016, 11, e0157298, doi:10.1371/journal.pone.0157298.

68. Hajishafiee, M.; Elovaris, R.A.; Jones, K.L.; Heilbronn, L.K.; Horowitz, M.; Poppitt, S.D.; Feinle-Bisset, C. Effects of intragastric administration of L-tryptophan on the glycaemic response to a nutrient drink in men with type 2 diabetes—impacts on gastric emptying, glucoregulatory hormones and glucose absorption. Nutr. Diabetes 2021, 11, 1–9, doi:10.1038/s41387-020-00146-9.

69. Ji, K.; Liang, H.; Chisomo-Kasiya, H.; Mokrani, A.; Ge, X.; Ren, M.; Liu, B. Effects of dietary tryptophan levels on growth performance, whole body composition and gene expression levels related to glycometabolism for juvenile blunt snout bream, Megalobrama amblycephala. Aquac. Nutr. 2018, 24, 1474–1483, doi:10.1111/anu.12684.

70. Iynedjian, P.B. Molecular physiology of mammalian glucokinase. Cell. Mol. Life Sci. 2009, 66, 27–42, doi:10.1007/s00018-008-8322-9.

71. Zelent, B.; Odili, S.; Buettger, C.; Shiota, C.; Grimsby, J.; Taub, R.; Magnuson, M.A.; Vanderkooi, J.M.; Matschinsky, F.M. Sugar binding to recombinant wild-type and mutant glucokinase monitored by kinetic measurement and tryptophan fluorescence. Biochem. J. 2008, 413, 269–280, doi:10.1042/BJ20071718.

72. Ray, P.D.; Foster, D.O.; Lardy, H.A. Paths of carbon in gluconeogenesis and lipogenesis: IV. Inhibition by L-tryptophan of hepatic gluconeogenesis at the level of phosphoenolpyruvate formation. J. Biol. Chem. 1966, 241, 3904–3908, doi:10.1016/S0021-9258(18)99792-9.

73. Foster, D.O.; Ray, P.D.; Lardy, H.A. A paradoxical in vivo effect of L-tryptophan on the phosphoenolpyruvate carboxykinase of rat liver. Biochemistry 1966, 5, 563–569, doi:10.1021/bi00866a023.

74. Rippe, D.F.; Berry, L.J. Effect of endotoxin on the activation of phosphoenolpyruvate carboxykinase by tryptophan. Infect. Immun. 1972, 6, 97–98, doi:10.1128/IAI.6.1.97-98.1972.

75. Quinn, P.G.; Yeagley, D. Insulin regulation of PEPCK gene expression: A model for rapid and reversible modulation. Curr. Drug Targets-Immune. Endocr. Metabol. Disord. 2005, 5, 423–437, doi:10.2174/156800805774912962.

76. Karim, S.; Adams, D.H.; Lalor, P.F. Hepatic expression and cellular distribution of the glucose transporter family. World J. Gastroenterol. 2012, 18, 6771–6781, doi:10.3748/wjg.v18.i46.6771.

77. Eleftheriadis, T.; Pissas, G.; Sounidaki, M.; Tsogka, K.; Antoniadis, N.; Antoniadi, G.; Liakopoulos, V.; Stefanidis, I. Indoleamine 2, 3-dioxygenase, by degrading L-tryptophan, enhances carnitine palmitoyltransferase I activity and fatty acid oxidation, and exerts fatty acid-dependent effects in human alloreactive CD4+ T-cells. Int. J. Mol. Med. 2016, 38,1605–1613, doi:10.3892/ijmm.2016.2750.

78. Sun, H.; Huang, F.F.; Qu, S. Melatonin: A potential intervention for hepatic steatosis. Lipids Health Dis. 2015, 14, 1–6, doi:10.1186/s12944-015-0081-7.

79. Nishida, S.; Segawa, T.; Murai, I.; Nakagawa, S. Long-term melatonin administration reduces hyperinsulinemia and improves the altered fatty-acid compositions in type 2 diabetic rats via the restoration of Δ-5 desaturase activity. J. Pineal Res. 2002, 32, 26–33, doi:10.1034/j.1600-079x.2002.10797.x.

80. Gao, J.; Xu, K.; Liu, H.; Liu, G.; Bai, M.; Peng, C.; Li, T.; Yin, Y. Impact of the gut microbiota on intestinal immunity mediated by tryptophan metabolism. Front. Cell. Infect. Mi. 2018, 8, 13, doi:10.3389/fcimb.2018.00013.

81. Yu, Y.; Raka, F.; Adeli, K. The role of the gut microbiota in lipid and lipoprotein metabolism. J. Clin. Med. 2019, 8, 2227, doi:10.3390/jcm8122227.

82. Utzschneider, K.M.; Kratz, M.; Damman, C.J.; Hullarg, M. Mechanisms linking the gut microbiome and glucose metabolism. J. Clin. Endocrinol. Metab. 2016, 101, 1445–1454, doi:10.1210/jc.2015-4251.

83. Wenninger, J.; Meinitzer, A.; Holasek, S.; Schnedl, W.J.; Zelzer, S.; Mangge, H.; Herrmann, M.; Enko, D. Associations between tryptophan and iron metabolism observed in individuals with and without iron deficiency. Sci. Rep. 2019, 9, 1–9, doi:10.1038/s41598-019-51215-8.

84. Wessels, A.G.; Kluge, H.; Hirche, F.; Kiowski, A.; Bartelt, J.; Corrent, E.; Stangl, G.I. High leucine intake reduces the concentration of hypothalamic serotonin in piglets. J. Anim. Sci. 2016, 94, 26–29, doi:10.2527/jas.2015-9728.

85. van der Hoek, G.A.; Cooper, S.J. Ondansetron, a selective 5-HT3 receptor antagonist, reduces palatable food consumption in the nondeprived rat. Neuropharmacology 1994, 33, 805–811, doi:10.1016/0028-3908(94)90120-1.

86. Yabut, J.M.; Crane, J.D.; Green, A.E.; Keating, D.J.; Khan, W.I.; Steinberg, G.R. Emerging roles for serotonin in regulating metabolism: New implications for an ancient molecule. Endocr. Rev. 2019, 40, 1092–1107, doi:10.1210/er.2018-00283.

87. Namkung, J.; Kim, H.; Park, S. Peripheral serotonin: A new player in systemic energy homeostasis. Mol. Cell. 2015, 38, 1023–1028, doi:10.14348/molcells.2015.0258.

88. Schoenichen, C.; Bode, C.; Duerschmied, D. Role of platelet serotonin in innate immune cell recruitment. Front. Biosci. 2019, 24, 514–526, doi:10.2741/4732.

89. Christensen, R.D.; Henry, E.; Wiedmeier, S.E.; Stoddard, R.A.; Sola-Visner, M.C.; Lambert, D.K.; Kiehn, T.I.; Ainsworth, S. Thrombocytopenia among extremely low birth weight neonates: Data from a multihospital healthcare system. J. Perinatol. 2006, 26, 348–353, doi:10.1038/sj.jp.7211509.

CHAPTER IV

EFFECT OF ISOLEUCINE AND ADDED VALINE ON PERFORMANCE, NUTRIENTS DIGESTIBILITY AND GUT MICROBIOTA COMPOSITION OF PIGS FED WITH VERY LOW PROTEIN DIETS

This chapter is based on: Parniyan Goodarzi, Caitlyn Marie Wileman1, Mohammad Habibi, Katherine Walsh, Julia Sutton, Cedrick Ndhumba Shili, Jianmin Chai, Jiangchao Zhao, and Adel Pezeshki. Effect of Isoleucine and Added Valine on Performance, Nutrients Digestibility and Gut Microbiota Composition of Pigs Fed with Very Low Protein Diets. International Journal of Molecular Sciences, Accepted.

4.1. Abstract

Little is known whether a combination Ile and added Val improves the growth of pigs offered with very low protein (VLP) diets through changes in nutrients digestibility and gut microbiota. The objective of this study was to investigate the effect of a mixture of Val above and Ile at NRC levels on growth, nutrient digestibility and gut microbiota in pigs fed with VLP diets. Forty, weaned piglets were assigned to: positive control: normal-protein-diet; negative control (NC): VLP diet supplemented with first four limiting amino acids; VA: NC with Val above NRC; IL: NC with Ile at NRC level; VAIL: NC with Val above and Ile at NRC levels. While both VAIL and VA groups completely recovered the inhibitory effects of VLP-diets o feed intake, only VAIL partially recovered the negative effects of VLP-diets on growth performance.

VAIL and VA increased the thermal radiation and decreased the digestibility of nitrogen. NC increased the relative abundance of Pasteurellaceae and Enterobacteriacea in the colon. VAIL had higher abundance of colonic Actinobacteria, Enterococcus, and Brevibacillus and the colon content of VA was more enriched with Mogibacterium. Overall, VAIL partially improved the growth performance which is likely linked with alterations in gut microbiota composition.

4.2. Introduction

Due to their negative impact on the environment, diet cost, post-weaning diarrhea and human and animal health, high protein diets have been criticized to be used for swine [1]. Slightly low protein (SLP) diets with less than 4% reduced crude protein (CP) supplemented with first four limiting amino acids (FFL), i.e., lysine (Lys), methionine (Met), threonine (Thr) and tryptophan (Trp) reduce nitrogen (N) excretion, and post-weaning diarrhea and improve gut health [2,3] with no negative influence on growth performance of pigs [4–7]. Reduction of dietary CP more than 4% may produce even more beneficial results in total N excretion [8–10], but very low protein (VLP) diets reduce the growth performance of pigs while supplemented with FFL [11–14]. Further research is warranted to identify the next limiting amino acids (AA) in pigs fed with VLP diets.

We have previously shown that supplementing VLP diets with all three branched-chain amino acids (BCAA) including leucine (Leu), isoleucine (Ile), and valine (Val) at or above NRC (2012) [15] levels along with adding FFL partially reversed the negative effect of these diets on growth of pigs [16,17]. Val and Ile have already been recommended as the fifth and sixth limiting AA for growth respectively in growing-finishing pigs fed with VLP diets [18,19]. Valine deficiency has been shown to reduce the feed intake in 6-week-old pigs fed with VLP diet supplemented with FFL and histidine [20]. The reduction in growth following Val deficiency was more severe when these diets were supplemented with the excess amount of Leu [21]. Increasing dietary Leu levels has been shown to decrease daily feed intake and growth in weaned pigs fed with normal protein diets

[22,23]. Therefore, dietary Val and Ile, but not Leu appear to have some promising effects on feed intake and growth of pigs under protein restriction.

We recently showed that supplementing a combination of Val and Ile at NRC (2012) [15] levels to VLP diets containing FFL improved growth performance of nursery pigs [24]. Others showed that dietary Val, and/or a combination of Val and Ile at NRC levels improved feed intake, growth performance and/or feed efficiency of pigs when added to both SLP [2,25–27] and VLP [18,28–33] diets containing FFL. However, adding Ile alone at NRC [15] levels to SLP and VLP diets supplemented with FFL either did not enhance the growth performance [2,25,28] or reduced feed intake and growth efficiency [18,29] in pigs. To our knowledge no study has examined the effect of a mixture of dietary Val above NRC [15] levels and standard amount of Ile on growth of pigs offered with VLP diets.

Dietary BCAA enhance growth likely by improving intestinal development [24,34–36], feed intake [17,26,37–39], nutrients digestibility [40,41], AA utilization [42], insulin-like growth factor 1 (IGF-1) signaling [17], microbiota composition [43,16] and muscle protein synthesis [26,44–46]. The underlying mechanisms by which dietary Val, Ile and/or combination of both improve growth performance in pigs fed with VLP diets is poorly understood. Therefore, the objective of this study was to investigate the effect of Val above and Ile at NRC (2012) [15] levels on growth measurements, nutrient digestibility and gut microbiota in pigs fed with VLP diets.

4.3. Methods

4.3.1. Animals and housing

All the experimental procedures used in this study were approved by Institutional Animal Care and Use Committee (IACUC-20-54) at Oklahoma State University. Forty weaned barrows (Duroc sire line and Large White × Landrace dam) weighing on average 6.14 kg at 3 weeks of age (Seaboard, Hennessey, OK) were individually housed in a controlled temperature facility. The room

temperature was set at 30 °C in the first week and then gradually decreased to 26 °C in the last week of the study. Every pen was equipped with a single-hole stainless steel feeder and cup waterers (Aqua Chief, Newton Grove, NC) attached to calibrated buckets with a single ½″ nipple (Lixit Nipple Waterer – L-70, Newton Grove, NC). The lightening program was scheduled based on 12 h light and 12 h half-light (with lights on at 0800 and off at 2000). Throughout the study, all pigs had ad libitum access to feed and water.

4.3.2. Diets and experimental design

Following 1 week of adaptation, weight matched pigs with an average body weight (BW) of 6.68 kg were randomly assigned to 5 dietary treatments for 5 weeks as follows: 1) positive control (PC): normal protein diet; 2) negative control (NC): very low protein diet with lysine, methionine, threonine and tryptophan (first four limiting AA, LAA) at NRC (2012) [15] levels; 3) VA: NC with Val above NRC level; 4) IL: NC with Ile at NRC level; 5) VAIL: NC with Val above and Ile at NRC level. National Swine Nutrition Guide (Version 2.1 Metric, ©2012 U.S. Pork Center of Excellence) was used for diet formulation. According to NRC recommendations, 3 nursery phase diets were prepared to ensure nutrient requirement of animals are met. Nursery phase 1 (N1), phase 2 (N2) and phase 3 (N3) were fed on days 1-7, 8-21 and 22-42, respectively. All diets were formulated to be isocaloric by using variable amounts of corn and soybean meal. Further, NC, Va, IL and VAIL diets were prepared isonitrogenous using L-Alanine. The amount of ingredients used was kept as consistent as possible among diets. The ingredients and chemical composition of diets are given in Table 4.1 and the analyzed chemical compositions of experimental diets are presented in Table 4.2.

Table 4.1. Experimental diets' ingredients and calculated chemical composition (as-fed basis)

	Diets[1]									
	N1	N2					N3				
Ingredients[2], %		**PC**	**NC**	**VA**	**IL**	**VAIL**	**PC**	**NC**	**VA**	**IL**	**VAIL**
Corn, yellow dent	36.98	46.35	67.62	67.49	67.50	67.35	66.20	84.04	83.93	83.94	83.82
Soybean meal, 47.5% CP	17.00	26.40	6.60	6.60	6.60	6.60	21.28	2.83	2.83	2.83	2.83
Fish meal, menhaden	6.00	3.20	3.20	3.20	3.20	3.20	3.20	3.20	3.20	3.20	3.20
Whey, dried	25.00	4.50	4.50	4.50	4.50	4.50	–	–	–	–	–
Corn starch	–	13.88	10.62	10.62	10.62	10.62	4.00	2.91	2.91	2.91	2.91
Lactose	7.00	–	–	–	–	–	–	–	–	–	–
Plasma spray-dried	5.80	2.10	2.10	2.10	2.10	2.10	2.10	2.10	2.10	2.10	2.10
Corn oil	0.37	–	–	–	–	–	–	–	–	–	–
Dicalcium phosphate 18.5%	0.85	1.35	1.73	1.73	1.73	1.73	1.15	1.55	1.55	1.55	1.55
Limestone	0.39	0.50	0.41	0.41	0.41	0.41	0.47	0.34	0.34	0.34	0.34
Salt	0.13	0.59	0.59	0.59	0.59	0.59	0.48	0.48	0.48	0.48	0.48
Chromium oxide	–	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Trace mineral premix	–	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Zinc oxide, 72% Zn	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
L-Lysine, HCl	0.26	0.35	0.85	0.85	0.85	0.85	0.36	0.83	0.83	0.83	0.83
DL-methionine	0.13	0.11	0.20	0.20	0.20	0.20	0.09	0.17	0.17	0.17	0.17
L-threonine	0.04	0.10	0.37	0.37	0.37	0.37	0.10	0.36	0.36	0.36	0.36
L-tryptophan	–	0.01	0.11	0.11	0.11	0.11	0.01	0.10	0.10	0.10	0.10
L-isoleucine	–	–	–	–	0.33	0.33	–	–	–	0.31	0.31
L-valine	–	–	–	0.48	–	0.48	–	–	0.44	–	0.44
L-alanine	–	–	0.54	0.19	0.33	–	–	0.53	0.20	0.32	–
Calculated Chemical Composition[3]											
Dry matter, %	90.38	90.36	90.14	90.05	90.16	90.18	88.93	88.97	88.98	88.98	88.99
ME, Mcal/kg	3.40	3.40	3.40	3.40	3.40	3.40	3.33	3.34	3.34	3.34	3.34
Crude protein, %	21.97	20.30	14.00	14.00	14.00	14.00	18.93	12.93	12.93	12.93	12.93
Crude fiber, %	1.61	2.23	1.86	1.85	1.85	1.85	2.47	2.07	2.07	2.07	2.06
Crude fat, %	3.23	2.72	3.08	3.08	3.08	3.08	3.28	3.54	3.54	3.54	3.53

Calcium, %	0.85	0.80	0.80	0.80	0.80	0.80	0.70	0.70	0.70	0.70	0.70
Total phosphorus, %	0.70	0.65	0.65	0.65	0.65	0.65	0.60	0.60	0.60	0.60	0.60
Available phosphorus, %	0.62	0.46	0.51	0.51	0.51	0.51	0.39	0.44	0.44	0.44	0.44
SID Lysine, %	1.50	1.35	1.35	1.35	1.35	1.35	1.23	1.23	1.23	1.23	1.23
SID Threonine, %	0.88	0.79	0.79	0.79	0.79	0.79	0.73	0.73	0.73	0.73	0.73
SID Methionine, %	0.43	0.39	0.39	0.39	0.39	0.39	0.36	0.36	0.36	0.36	0.36
SID Tryptophan, %	0.25	0.22	0.22	0.22	0.22	0.22	0.20	0.20	0.20	0.20	0.20
SID Isoleucine, %	0.78	0.74	0.41	0.41	0.74	0.74	0.67	0.36	0.36	0.67	0.67
SID Valine, %	0.96	0.86	0.53	1.01	0.53	1.01	0.79	0.48	0.92	0.48	0.92
SID Leucine, %	1.65	1.50	1.05	1.05	1.05	1.05	1.46	1.03	1.03	1.03	1.02
SID Histidine, %	0.51	0.47	0.30	0.30	0.30	0.30	0.45	0.28	0.28	0.28	0.28
SID Arginine, %	1.13	1.19	0.62	0.62	0.62	0.62	1.09	0.55	0.55	0.55	0.55
SID Phenylalanine, %	0.90	0.86	0.51	0.51	0.51	0.51	0.80	0.47	0.47	0.47	0.47
SID Valine: SID Lysine	0.64	0.64	0.39	0.75	0.39	0.75	0.64	0.39	0.75	0.39	0.75
SID Isoleucine: SID Lysine	0.52	0.55	0.30	0.30	0.55	0.55	0.54	0.29	0.29	0.54	0.54

[1]National Swine Nutrition Guide (NSNG; Version 2.1 Metric, ©2012 U.S. Pork Center of Excellence) was used for diets formulations. PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (i.e., lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; VA: NC containing valine (Val) above of NRC level; IL: NC containing isoleucine (Ile) at NRC level; VAIL: NC containing Val above NRC and Ile at NRC levels. N1: nursery phase 1 diet offered from days 1 to 7 of the experiment, N2: nursery phase 2 diets fed from days 8 to 21 of the experiment and N3: nursery phase 3 diets offered from days 22 to 42 of the experiment.

[2]Corn, soybean meal, fish meal, whey, corn starch, lactose, plasma spray-dried, corn oil, dicalcium phosphate, limestone, salt, zinc oxide, DL-methionine (99%) and L-lysine HCl (79-99%) were obtained by Nutra Blend, LLC (Neosho, MO). L-threonine (98.5%) and L-tryptophan (98%) were purchased from Ajinomoto (Overland Park, KS). L-isoleucine (98.5%), L-alanine and L-valine (96.5%) were provided from Ajinomoto Health & Nutrition North America, Inc. (Raleigh, NC). Chromium oxide was ordered from Fisher Scientific (Bartlesville, OK). Vitamin premix was obtained from Nutra Blend, LLC (Neosho, MO): vitamin A, 1,653,750 IU/kg; vitamin D3, 661,500 IU/kg; vitamin E, 17,640 IU/kg; vitamin K (menadione), 1,323 mg/kg; vitamin B12, 13.23 mg/kg; niacin, 19,845 mg/kg;

D-pantothenic acid, 11,025 mg/kg; riboflavin, 3,307.5 mg/kg; phytase, 300,056.4 FYT/kg. Trace mineral premix was obtained from Nutra Blend, LLC (Neosho, MO): copper, 11,000 ppm; iodine, 198 ppm; iron, 73,000 ppm; manganese, 22,000 ppm; selenium, 198 ppm; zinc, 73,000 ppm.

[3]ME: Metabolize energy; SID: Standard Ileal Digestibility

Table 4.2. Analyzed chemical composition of experimental diets (as-fed basis)

Chemical composition	Diets[1]									
	N1	N2					N3				
		PC	NC	VA	IL	VAIL	PC	NC	VA	IL	VAIL
Dry matter, %	90.60	88.10	87.60	86.80	87.70	87.00	87.30	86.80	86.60	86.90	86.50
Crude protein, %	23.00	20.00	13.70	13.50	14.40	13.50	18.70	13.20	12.80	12.40	13.20
Crude fiber, %	1.40	2.00	1.70	1.80	1.90	1.40	2.90	2.00	1.90	1.90	2.00
Calcium, %	1.06	0.92	0.66	0.83	0.88	0.78	0.78	0.64	0.66	0.71	0.67
Phosphorus, %	0.94	0.76	0.67	0.74	0.79	0.70	0.61	0.61	0.67	0.69	0.69
Taurine[2], %	0.20	0.19	0.28	0.19	0.20	0.20	0.26	0.28	0.21	0.21	0.20
Hydroxyproline, %	0.11	0.09	0.05	0.06	0.10	0.08	0.06	0.06	0.09	0.06	0.06
Aspartic acid, %	2.10	2.09	1.02	1.06	1.30	0.93	1.81	0.88	0.89	0.94	0.97
Threonine, %	1.06	0.93	0.85	0.75	0.92	0.80	0.86	0.75	0.72	0.94	0.98
Serine, %	0.95	0.91	0.51	0.51	0.59	0.47	0.81	0.47	0.47	0.49	0.50
Glutamic acid, %	3.45	3.57	1.94	2.03	2.41	1.85	3.31	1.80	1.79	1.86	1.89
Proline, %	1.17	1.11	0.73	0.76	0.81	0.72	1.05	0.72	0.76	0.76	0.77
Lanthionine[2], %	0.02	0.01	0.02	0.00	0.00	0.00	0.02	0.02	0.00	0.00	0.00
Glycine, %	0.95	0.88	0.53	0.52	0.63	0.49	0.83	0.51	0.52	0.53	0.51
Alanine, %	1.14	1.02	1.29	0.97	1.08	0.69	1.00	1.19	0.84	1.21	0.72
Cysteine, %	0.44	0.39	0.23	0.24	0.29	0.21	0.33	0.23	0.20	0.24	0.26
Valine, %	1.18	1.04	0.61	1.03	0.74	1.04	0.94	0.55	0.98	0.60	0.97
Methionine, %	0.47	0.44	0.36	0.35	0.44	0.39	0.39	0.37	0.29	0.34	0.38
Isoleucine, %	0.93	0.91	0.49	0.50	0.89	0.75	0.81	0.43	0.41	0.74	0.71
Leucine, %	1.89	1.73	1.12	1.17	1.29	1.12	1.63	1.09	1.10	1.12	1.14
Tyrosine, %	0.73	0.68	0.39	0.39	0.47	0.39	0.63	0.39	0.39	0.38	0.39
Phenylalanine, %	1.01	1.02	0.56	0.60	0.69	0.54	0.92	0.53	0.54	0.53	0.56
Hydroxylysine, %	0.06	0.04	0.00	0.03	0.04	0.03	0.00	0.00	0.04	0.02	0.04
Ornithine[2], %	0.01	0.02	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01
Lysine, %	1.69	1.69	1.39	1.32	1.56	1.31	1.40	1.29	1.37	1.40	1.29
Histidine, %	0.54	0.54	0.32	0.32	0.37	0.28	0.51	0.30	0.30	0.31	0.30
Arginine, %	1.19	1.29	0.65	0.65	0.81	0.56	1.16	0.58	0.59	0.61	0.60
Tryptophan, %	0.33	0.28	0.23	0.25	0.26	0.28	0.22	0.20	0.22	0.24	0.22

Valine: Lysine	0.70	0.62	0.44	0.78	0.47	0.79	0.67	0.43	0.72	0.43	0.75
Isoleucine: Lysine	0.55	0.54	0.35	0.38	0.57	0.57	0.58	0.33	0.30	0.53	0.55

[1]PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (i.e., lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; VA: NC containing valine (Val) above of NRC level; IL: NC containing isoleucine (Ile) at NRC level; VAIL: NC containing Val above NRC and Ile at NRC levels. N1: nursery phase 1 diet offered from days 1 to 7 of the experiment, N2: nursery phase 2 diets fed from days 8 to 21 of the experiment and N3: nursery phase 3 diets offered from days 22 to 42 of the experiment.

[2]Non-proteinogenic amino acids.

4.3.3. Body weight, feed intake, and water intake

Body weight were recorded weekly and individual feed intake (FI) and water intake (WI) were measured daily. Using BW, FI and WI data, average daily gain (ADG), average daily feed intake (ADFI), average daily protein intake (ADPI), average daily water intake (ADWI), gain-to-feed ratio (G:F), gain-to-protein ratio (G:P) and water-to-feed ratio (W:F) were determined. Furthermore, weekly body weight gain (BWG), mean feed intake (MFI), cumulative feed intake (CFI), cumulative protein intake (CPI), G:F, and G:P ratios were calculated.

4.3.4. Thermal images

Using a FLIR C2 compact thermal camera with a focal length of 1.54 mm and a thermal accuracy of ±2 ºC (FLIR Systems, Boston, MA, USA), thermal images were captured approximately 1 m above each pig on a weekly basis with emissivity coefficient set at 0.95. Representative thermal images of experimental groups are shown in Supplementary Fig. 4A.1.

4.3.5. Feed and fecal samples collection

Roughly1 kg feed samples from each feed bag were collected, pooled for each treatment, and stored at −20 °C until composition analysis. Fecal samples were collected in plastic bags for individual pigs over the course of study, combined separately during N2 and N3 phases for each treatment and stored at −20 °C for composition analysis.

4.3.6. Blood and tissue samples collection

At week 6, after an overnight fast (8 hours), pigs were allowed to consume their respective diets for one hour and blood samples were collected at baseline 0 (fast state), 60 and 120 minutes after the meal test (fed state). Blood samples were collected from jugular vein in the supine position into 10.0 mL serum tubes and 3.0 mL plasma tubes containing heparin (BD Vacutainer®, Franklin Lakes, NJ, USA). Blood samples were centrifuged at 3,000 × g for 15

minutes (min) at 4 ºC and serum or plasma were separated. Both serum and plasma samples were stored at −80°C for further analysis. At 120 min after meal, all pigs were euthanized using CO2 asphyxiation method. Immediately after euthanasia colon content were collected, snap-frozen in liquid nitrogen, and stored at −80 °C for microbiota analyses. One of back legs was excised and stored at −20 °C for dual-energy X-ray absorptiometry (DEXA) analysis.

4.3.7. Diets and fecal samples composition analysis

Diets were analyzed for dry matter, CP, crude fiber, calcium (Ca), phosphorus (P), and chromium (Cr), by ServiTech laboratories (Dodge City, KS) as we previously described [13,17,47] (Table 4.1). Experimental diets were also analyzed for complete AA profile by Agricultural Experiment Station Chemical Laboratories (University of Missouri-Columbia, MO) [48] (Table 4.2). N, Ca, P and Cr contents of fecal samples were determined by ServiTech laboratories (Dodge City, KS, USA).

4.3.8. Thermal radiation analysis

A rectangle shape was drawn in the entire back of piglets using FLIR camera software (FLIR Research Studio software) to determine the mean dorsal surface body temperature [48]. Following equation was then used to obtain thermal radiation (W/m2): σε (Ts4 – Tα4) where σ is Stefan Boltzmann constant (5.67 x 10-8 W/m2K4), ε is thermodynamic emissivity (0.95), Ts is body surface temperature (kelvin) and Tα is ambient temperature (kelvin).

4.3.9. Apparent fecal digestibility

Marker method was used for calculating apparent fecal digestibility (AFD) of Ca, P, and N by comparing the difference between the quantities of Cr and nutrients in feed and feces for individual pigs using the following formula: AFD = 100 – [(100 × (Cr concentration in feed/Cr concentration in feces) × (nutrient concentration in feces/nutrient concentration in feed)] [47].

4.3.10. Dual-energy X-ray absorptiometry analysis

Using rodent's calibration feature, excised legs were scanned with DEXA (Hologic, Discovery QDR Series, Bedford, MA, USA) to obtain bone mineral content (BMC), bone mineral density (BMD), fat mass, lean mass, and total mass [47].

4.3.11. Colon contents DNA isolation, amplicon sequencing, sequence data analysis, and taxonomic classification

The DNA from colon contents were extracted using the Dneasy PowerLyzer PowerSoil Kit (Qiagen, Inc., Germantown, MD, USA) following the instructions of the manufacturer. The concentration of isolated DNA was determined using NanoDrop One (Thermo Fisher Scientific, Madison, WI, USA) and diluted to 10 ng/μL. The DNA samples with OD 260/280 of 1.8–2 were used for PCR amplification and microbial amplicon sequencing.

For amplicon sequencing, the primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) with barcodes were used for amplifying the 16S rRNA V4 region by PCR. The PCR products was mixed with 1× loading buffer containing GelRed and loaded on 1% electrophoresis agarose gel for quality control and then purified using normalization plates (SequalPrep Normalization Plate Kit, Invitrogen, Carlsbad, CA, USA). Purified PCR amplicons were pooled together to generate a sequencing library. The library quality and concentration were determined by using the Agilent Bioanalyzer 2100 system (Agilent, Santa Clara, CA, USA) and KAPA Illumina Library Quantification Kits (Roche, Indianapolis, IN, USA). A mock community (ZymoBIOMICS™ Microbial Community Standard, Zymo, Irvine, CA, USA) and negative control were included in the sequencing run for quality control to estimate errors introduced during PCR amplification and the MiSeq run. The library was then sequenced using the Illumina MiSeq platform (Illumina, Inc.), and 2 × 250 bp paired-end raw reads were generated.

For sequence data analysis, the Mothur software (v1.39.1) [49] was used to process the raw paired-end reads based on the MiSeq SOP. Paired-end reads were merged and filtered. Chimeras was removed by the UCHIME algorithm. Sequences were binned into operational taxonomic units (OTUs) at the 97% similarity level and be classified using a naïve Bayesian classifier against Ribosomal Database Project (RDP)[50]. High quality reads were classified against the RDP database. Alpha diversities including Shannon index, the number of Observed OTUs, Chao1 and Simpson were compared using Wilcoxon rank test. Beta diversity based on Bray–Curtis and Jaccard distances was tested using an analysis of similarity (ANOSIM). The outputs of diversity were visualized using the "ggplot2" package in R (version 3.6.0). To identify the most notable bacterial communities between dietary groups, linear discriminant analysis (LDA) with effect size measurements (LefSe) was applied using a tool hosted in the Galaxy (server) instance of Huttenhower lab (https://huttenhower.sph.harvard.edu/galaxy/) and the scores were normalized by log10. The bacterial populations with LDA score (log10) > 2 were considered as significantly increased numbers.

4.3.12. Statistical analysis

Outlier test was first conducted for overall growth, cumulative hourly feed intake, thermal radiation, and all other data acquired from laboratory analyses, including DEXA. GLM analysis (IBM SPSS Statistics Version 23, Armonk, NY, USA) and a paired Student's t-test followed by a Benjamini-Hochberg correction with 0.1 false discovery rate was then used to determine the differences among means for five preplanned comparisons: NC vs. PC, VA vs. PC, VA vs. NC, VAIL vs. PC, and VAIL vs. NC. The mixed analysis was performed for the hourly, daily, and weekly collected data, including FI, WI, BW, BWG, MFI, CFI, CPI, G:F, and G:P, with the diet, time, and diet by time interaction as fixed effects and the animal as a random variable in the model. The modeling of covariance structure for repeated measurements for each variable was conducted using the lowest quantities of fit statistics for

corrected Akaike Information Criterion and Bayesian Information Criterion. Differences among treatments were considered significant at $P \leq 0.05$ and a trend at $0.05 < P \leq 0.10$.

4.4. Results

4.4.1. Growth performance

Overall, the effect of diet on final BW, ADG, ADFI, ADPI and G:F ratio was significant ($P < 0.05$, Table 4.3). Compared to PC, final BW of NC was reduced by 25%, while that tended ($0.05 < P \leq 0.1$) to be reduced in VA and VAIL (Table 4.3). Relative to NC, VAIL, but not VA, tended to increase the final BW (Table 4.3). Pigs fed with NC, VA, and VAIL had 36, 24, and 22% lower ADG in comparison with PC, respectively. VAIL tended to increase the ADG compared to NC (Table 4.3). Body length, heart girth and wither height were not changed across diets (Table 4.3). Overall, the effects of diet, day, and interaction of diet by day on daily BW were significant ($P < 0.01$; Fig. 4.1A). NC had lower BW than PC on days 21, 28, and 35 (22, 22, and 25%, respectively (Fig. 4.1A). Relative to PC, VA tended to have a lower BW on days 21, 28, and 35; however, VA tended to have a higher BW than NC on day 21. VAIL tended to reduce BW compared to PC on days 28 and 35 but this group tended to have higher BW than NC on day 35 (Fig. 4.1A). Further, the effect of diet on BWG was significant for weeks 2-5 and tended to be significant for week 1 (Table 4.4). Compared to PC, pigs fed with NC had a lower BWG during all 5 weeks (26, 38, 41, 37, and 38%, respectively). In comparison with PC, pigs fed with VA either tended or had significantly lower BWG in all 5 weeks. Pigs fed with VA had higher BWG than NC in week 3 (Table 4.4). VAIL tended to have a lower BWG than PC in weeks 2 and 5 and had lower (38%) BWG in week 3. However, compared to NC, VAIL had higher (40%) and tended to have higher BWG during weeks 4 and 5, respectively. Pigs in NC group had a lower ADFI than PC, but the ADFI was not different when VA and VAIL were groups compared with PC (Table 4.3). Compared to NC, the ADFI was 31% higher in VA and tended to be higher in VAIL (Table

4.3). ADWI and W:F were not changed among diets (Table 4.3). The effect of diet on FI was significant on day 4 with a lower FI for VAIL than PC and NC at hour 9 ($0.05 < P \leq 0.1$; Supplementary Fig. 4A.2). Overall, the effect of diet on MFI and CFI was significant in weeks 3-5 (Table 4.4). Relative to PC, NC tended to reduce MFI and CFI in week 3. VA tended to increase MFI and CFI compared to NC in week 3. NC had a lower MFI and CFI than PC in week 4. VA had a higher MFI and CFI than NC in week 4 and VAIL tended to have a higher MFI and CFI than NC in week 4 (Table 4.4).

ADPI was lower in NC, VA, and VAIL (67, 27, and 50%, respectively) compared to PC (Table 4.3). Furthermore, ADPI was 38% higher in VA and tended to be higher in VAIL compared to NC (Table 4.3). Overall, the effect of diet on CPI was significant over the course of 5 weeks (Table 4.4). In all weeks, pigs fed with NC had lower CPI than PC. Except week 2, pigs fed with VA had a lower CPI than PC in weeks 1, 3, 4 and 5 (36, 33, 31, and 45%, respectively) (Table 4.4). However, compared to NC pigs fed with VA increased CPI by 24 and 34% in weeks 3 and 4, respectively. Similarly, except week 2, pigs fed with VAIL had a lower CPI than PC in weeks 1, 3, 4 and 5 (40, 41, 30, and 27%, respectively) while, in comparison with NC, VAIL had 36 and 32% higher CPI in weeks 4 and 5, respectively.

Compared to PC, pigs fed with NC, VA, and VAIL had 26, 29, and 29% lower G:F ratio, respectively (Table 4.3). The effect of diet on weekly G:F ratio was significant on weeks 3 and 4 (Table 4.4). Pigs fed with NC had a lower G:F than PC in weeks 2, 4, 5. Relative to PC, pigs fed with VA decreased the G:F by 24, 23, and 31%, in weeks 2, 3, and 4 respectively and tended to reduce the G:F on week 5. VA tended to have a lower G:F than NC on week 4. In the last 3 weeks, G:F ratio in pigs fed with VAIL was lower in comparison with PC (23, 14 and 19%, respectively). The G:P ratio was not different across diets (Table 4.3). The effect of diet on weekly G:P ratio was only significant on week 4 when pigs fed with VAIL had a higher (23%) G:P ratio than PC (Table 4.4).

Table 4.3. Growth performance of nursery pigs fed with very low-protein diets supplemented with isoleucine at NRC and valine above NRC levels, or a combination of the two.

Measurements[1]	Diets[1]					SEM[2]	*P*-value
	PC	NC	VA	IL	VAIL		
Initial BW, kg	6.76	6.69	6.89	6.68	6.68	0.12	0.98
Final BW, kg	23.92	17.98^{a}	20.26$^{\#}$	18.21	20.19$^{\varepsilon\omega}$	0.56	0.01
ADG, kg/day	0.50	0.32^{a}	0.38^{b}	0.33	0.39$^{d\omega}$	0.02	< 0.01
ADFI, kg/day	0.78	0.62^{a}	0.81^{c}	0.68	0.78$^{\omega}$	0.02	0.04
ADPI, kg/day	0.15	0.08^{a}	0.11^{bc}	0.09	0.10$^{d\omega}$	0.01	< 0.01
ADWI, L/day	1.95	1.38	1.73	1.55	1.77	0.08	0.24
G:F, kg/kg	0.66	0.49^{a}	0.47^{b}	0.48	0.50^{d}	0.02	< 0.01
G:P, kg/kg	3.47	3.66	3.62	3.73	3.74	0.09	0.91
W:F , L/kg	2.68	2.11	2.31	2.28	2.28	0.10	0.54
Final body length, m	0.65	0.61	0.65	0.61	0.63	0.01	0.08
Final heart girth, m	0.62	0.57	0.59	0.58	0.61	0.01	0.11
Final wither height, m	0.42	0.41	0.42	0.40	0.41	0.004	0.31

[1]PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (*i.e.*, lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; VA: NC containing valine (Val) above of NRC level; IL: NC containing isoleucine (Ile) at NRC level; VAIL: NC containing Val above NRC and Ile at NRC levels. BW: body weight; ADG: average daily gain; ADFI: average daily feed intake; ADPI: average daily protein intake; ADWI: average daily water intake; G:F: gain:feed ratio; G:P: gain:protein ratio; W:F: water:feed ratio. ^{a} $P \leq 0.05$ NC vs. PC, ^{b} $P \leq 0.05$ VA vs. PC, ^{c} $P \leq 0.05$ VA vs. NC, ^{d} $P \leq 0.05$ VAIL vs. PC, $^{\#}P \leq 0.1$ VA vs. PC, $^{\omega}$ $P \leq 0.1$ VAIL vs. NC, $^{\varepsilon}$ $P \leq 0.1$ VAIL vs. PC. The values are means, n=8.

[2]SEM: standard error of the mean.

Table 4.4. Growth performance of nursery pigs fed with very low-protein diets supplemented with isoleucine at NRC and valine above NRC levels, or a combination of the two.

	Diets[1]						
	PC	**NC**	**VA**	**IL**	**VAIL**	**SEM[2]**	***P*-value**
BWG[1], kg							
Week 1	1.48	1.09^{a}	1.07^{b}	1.32	1.09	0.06	0.08
Week 2	3.23	2.00^{a}	2.35$^{\#}$	1.82	2.19$^{\varepsilon}$	0.12	< 0.01
Week 3	4.11	2.44^{a}	3.05^{bc}	2.24	2.54^{d}	0.15	< 0.01
Week 4	4.33	2.74^{a}	3.05^{b}	2.56	3.83^{e}	0.16	< 0.01
Week 5	4.85	3.03^{a}	3.44^{b}	3.27	3.89$^{\varepsilon\omega}$	0.17	0.01
MFI[1], kg							
Week 1	0.44	0.40	0.42	0.35	0.42	0.02	0.54
Week 2	0.65	0.55	0.63	0.48	0.59	0.03	0.21
Week 3	0.83	0.63*	0.81$^{\delta}$	0.63	0.75	0.03	0.05
Week 4	0.96	0.73^{a}	0.96^{c}	0.72	0.94$^{\omega}$	0.03	< 0.01
Week 5	1.03	0.80	0.94	0.80	1.06	0.04	0.02
CFI[1], kg							
Week1	3.09	2.78	2.96	2.48	2.96	0.12	0.56
Week 2	4.56	3.86	4.40	3.34	4.15	0.21	0.20
Week 3	5.78	4.41*	5.65$^{\delta}$	4.41	5.28	0.19	0.05
Week 4	6.70	5.11^{a}	6.74^{c}	5.05	6.58$^{\omega}$	0.21	< 0.01
Week 5	7.22	5.58	6.59	5.63	7.44	0.25	0.02
CPI[1], kg							
Week 1	0.62	0.38^{a}	0.40^{b}	0.39	0.37^{d}	0.02	< 0.01
Week 2	0.91	0.53^{a}	0.65$^{\#\delta}$	0.51	0.66	0.04	< 0.01
Week 3	1.08	0.58^{a}	0.72^{bc}	0.55	0.64^{d}	0.04	< 0.01
Week 4	1.25	0.64^{a}	0.86^{bc}	0.63	0.87^{de}	0.05	< 0.01
Week 5	1.35	0.74^{a}	0.84^{b}	0.70	0.98^{de}	0.05	< 0.01
G:F[1], kg/kg							
Week 1	0.51	0.42	0.36	0.72	0.41	0.07	0.46
Week 2	0.72	0.53^{a}	0.55^{b}	0.59	0.76	0.06	0.65
Week 3	0.71	0.56*	0.55^{b}	0.50	0.55^{d}	0.02	0.01
Week 4	0.65	0.54^{a}	0.45$^{b\delta}$	0.51	0.56^{d}	0.02	< 0.01
Week 5	0.69	0.53^{a}	0.53$^{\#}$	0.60	0.56^{d}	0.02	0.11
G:P[1], kg/kg							
Week 1	2.54	3.06	2.69	3.06	3.29	0.12	0.31
Week 2	3.85	3.85	3.72	3.40	3.43	0.12	0.77
Week 3	3.82	3.79	4.27	4.05	4.20	0.11	0.56
Week 4	3.45	3.99	3.51	3.80	4.24^{d}	0.09	0.04
Week 5	3.70	3.71	4.13	4.55	4.41	0.13	0.13

[1]PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (*i.e.*, lysine, methionine, threonine, and tryptophan)

at NRC (2012) levels; VA: NC containing valine (Val) above of NRC level; IL: NC containing isoleucine (Ile) at NRC level; VAIL: NC containing Val above NRC and Ile at NRC levels. BWG: body weight gain; MFI: mean feed intake; CFI: cumulative feed intake; CPI: cumulative protein intake; G:F: gain:feed ratio; G:P: gain:protein ratio. [a] $P \leq 0.05$ NC vs. PC, [b] $P \leq 0.05$ VA vs. PC, [c] $P \leq 0.05$ VA vs. NC, [d] $P \leq 0.05$ VAIL vs. PC, [e] $P \leq 0.05$ VAIL vs. NC, [*] $P \leq 0.1$ NC vs. PC, [#] $P \leq 0.1$ VA vs. PC, [δ] $P \leq 0.1$ VA vs. NC, [ε] $P \leq 0.1$ VAIL vs. PC, [ω] $P \leq 0.1$ VAIL vs. NC. The *P*-values for the overall model effect for diet, week (wk) and diet × wk for BWG were < 0.01, < 0.01 and < 0.01, for MFI were < 0.01, < 0.01 and 0.169, for CFI were < 0.01, < 0.01 and 0.607, for CPI were < 0.01, < 0.01 and 0.025, for G:F were 0.180, 0.084 and 0.515 and for G:P were 0.092, < 0.01 and 0.427 respectively. The values are means, n=8.

[2]SEM: standard error of the mean.

4.4.2. Thermal radiation

The effect of diet on area under the curve (AUC) of thermal radiation was significant (P < 0.01; Fig. 4.1B). NC and VA tended to increase thermal radiation compared to PC. Pigs fed with VAIL had 22 and 12% higher AUC thermal radiation than PC and NC, respectively (Fig. 4.1B).

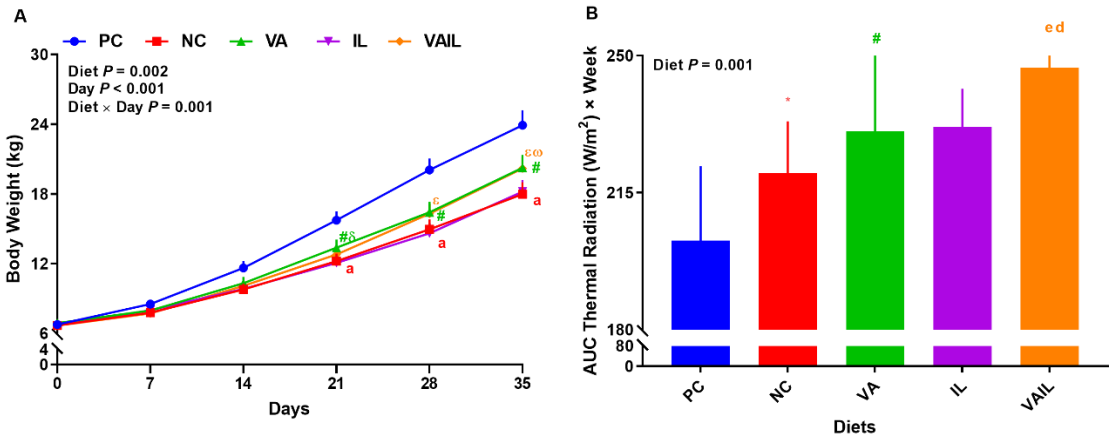


Figure 4.1. (A) Body weight, (B) area under the curve (AUC) for thermal radiation of nursery pigs fed with very low protein diets supplemented with isoleucine (Ile) at NRC and valine (Val) above NRC levels, or a combination of the two.

PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (i.e., lysine, methionine, threonine and tryptophan) at NRC (2012) levels; VA: NC containing Val above NRC level; IL: NC containing Ile at NRC level; VAIL: NC containing Val above NRC and Ile at NRC levels. ^{a} $P \leq 0.05$ NC vs. PC ^{d} $P \leq 0.05$ VAIL vs. PC, ^{e} $P \leq 0.05$ VAIL vs. NC, $^{*}P \leq 0.1$ NC vs. PC, $^{\#}P \leq 0.1$ VA vs. PC, $^{\delta}$ $P \leq 0.1$ VA vs. NC, $^{\varepsilon}$ $P \leq 0.1$ VAIL vs. PC, $^{\omega}$ $P \leq 0.1$ VAIL vs. NC. Values are means ± standard error of the mean. n=8.

4.4.3. Nutrients digestibility

There were no changes in AFD of Ca across dietary treatments (Fig. 4.2A). The overall effects of diet, phase, and diet by phase interaction on AFD of P were significant (Fig. 4.2B). Compared to PC, AFD of P was 15% higher in pigs fed with NC during N2 phase. The AFD of P in VA and VAIL was 6 and 16% lower than NC in N2 phase. In N3 phase, pigs fed with NC, VA, and VAIL had 14, 17 and 18% higher AFD of P than PC (Fig. 4.2B). Overall, the effect of diet and phase on AFD of N was significant (Fig. 4.2C). Pigs fed with VA reduced the AFD of N relative to NC in N2 and N3 phases. VA either tended to decrease or significantly decreased the AFD of N compared to PC in N2 and N3 phases, respectively. VAIL tended to have a lower AFD of N compared to NC in N2 phase (Fig. 4.2C).

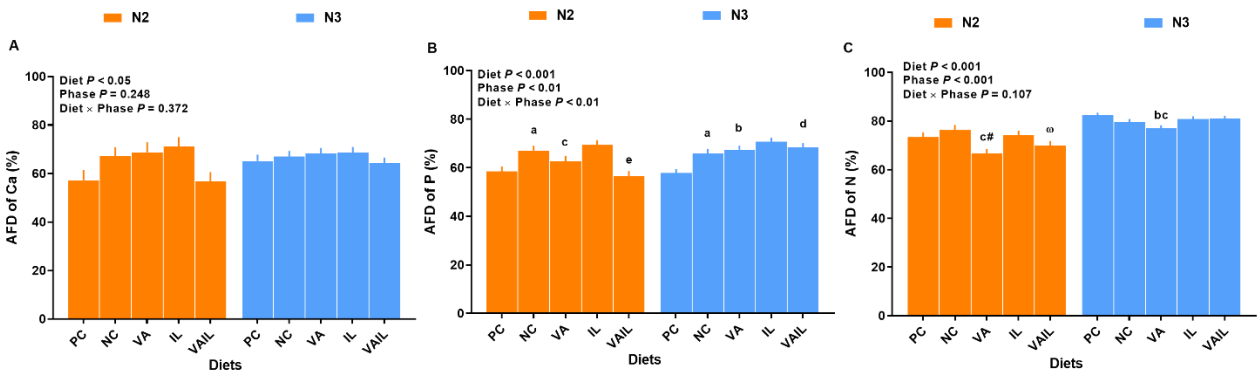


Figure 4.2. Apparent fecal digestibility (AFD) of (A) calcium (Ca), (B) phosphorus (P) and (C) nitrogen (N) in nursery pigs fed with very low protein diets supplemented with isoleucine (Ile) at NRC and valine (Val) above NRC levels, or a combination of the two.

PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (i.e., lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; VAIL: NC containing Val above NRC and Ile at NRC levels. [a] $P \leq 0.05$ NC vs. PC, [b] $P \leq$

0.05 VA vs. PC, [c] $P \leq 0.05$ VA vs. NC, [d] $P \leq 0.05$ VAIL vs. PC, [e] $P \leq 0.05$ VAIL vs. NC, [#]$P \leq 0.1$ VA vs. PC, [ω] $P \leq 0.1$ VAIL vs. NC. The values are means ± standard error of the mean. n=8.

4.4.4. Dual-energy X-ray absorptiometry analysis

Overall, the effect of diet on fat and lean percent was significant ($P \leq 0.01$; Table 4.5). In comparison with PC, pigs fed with VA tended to have a higher fat percent in leg samples. Relative to NC, VA tended to have a lower lean percent in their legs. BMC and BMD were not different across diets (Table 4.5).

Table 4.5. Dual-energy X-ray absorptiometry (DEXA) scan of nursery pigs' leg fed with very low-protein diets supplemented with isoleucine at NRC and valine above NRC levels, or a combination of the two

Measurements	Diets[1]						
	PC	NC	VA	IL	VAIL	SEM[2]	*P*-value
Fat, %	12.70	13.80	16.35[#]	19.69	14.77	0.62	< 0.01
Lean, %	84.97	83.71	81.01[δ]	77.99	82.94	0.64	< 0.01
BMC[1], g	47.09	39.65	45.78	39.10	41.23	1.58	0.38
BMD[1], g/cm^2	0.31	0.29	0.31	0.29	0.30	0.01	0.61

[1]PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (*i.e*., lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; VA: NC containing valine (Val) above of NRC level; IL: NC containing isoleucine (Ile) at NRC level; VAIL: NC containing Val above NRC and Ile at NRC levels. BMC: bone mineral content, BMD: bone mineral density. [#]$P \leq 0.1$ VA vs. PC, [δ] $P \leq 0.1$ VA vs. NC. The values are means, n=8.

[2]SEM: standard error of the mean.

4.4.5. Colon content microbiota

The rarefaction curve analysis showed that the species richness of all analyzed colon content samples reached a stable plateau at 30,000 reads and 400 OTUs indicating that there was a sufficient sequencing depth to saturate the bacterial populations in samples (Supplementary Fig. 4A.3 A-E). The alpha diversity metrics of bacterial community in each sample are shown in Fig. 4.3 A-D. Simpson index, which shows the species diversity was significantly different in VAIL compared to PC and VA ($P < 0.05$; Fig. 4.3B). No differences in Shannon, observed OTUs, and Chao1 were detected among groups (Fig. 4.3 A, C, and D).

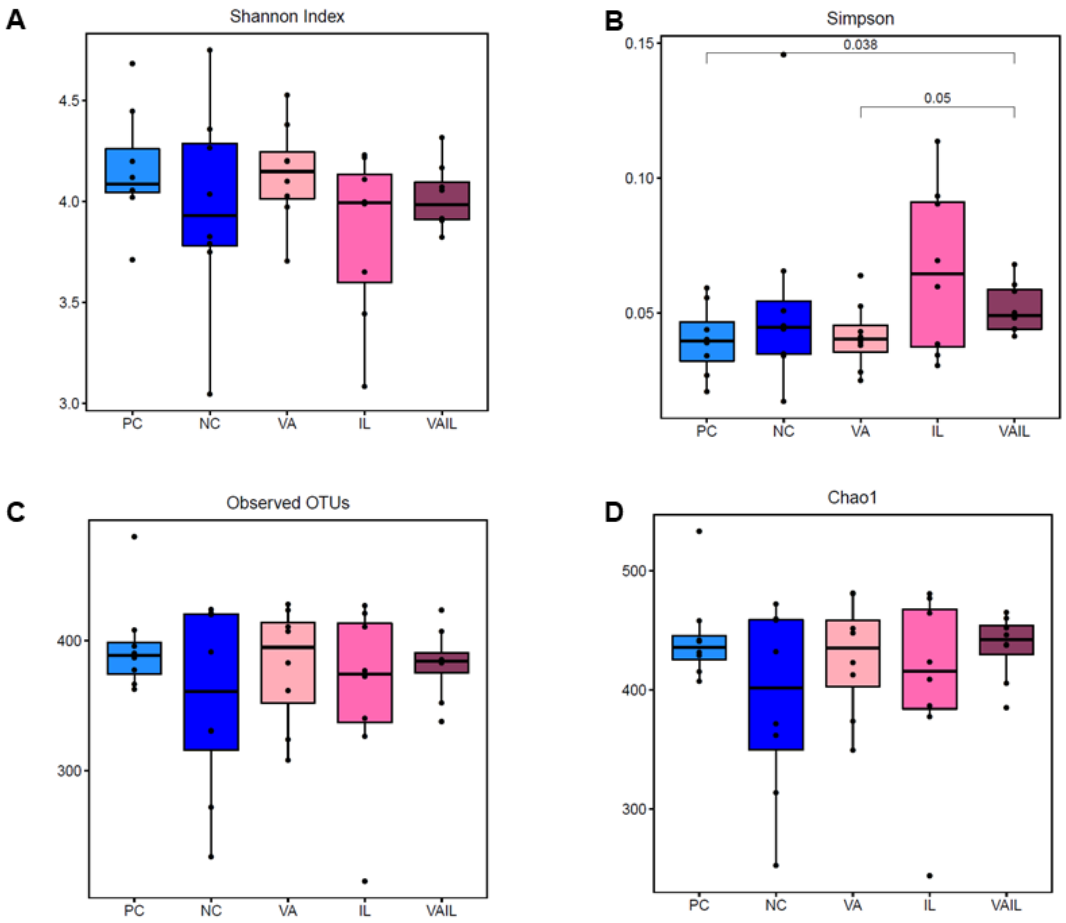


Figure 4.3. Alpha diversity indices for colon bacterial community in pigs fed with very low protein diets supplemented with isoleucine (Ile) at NRC and valine (Val) above NRC levels, or a combination of the two.

(A) Shannon, (B) Simpson, (C) Observed operational taxonomic units (OTUs), and (D) Chao1. PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (*i.e.*, lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; VA: NC containing Val above NRC level; IL: NC containing Ile at NRC level; VAIL: NC containing Val above NRC and Ile at NRC levels. Each node represents an individual pig. The means are different with $P \leq 0.05$. $n = 8$.

To beta diversity of the bacterial community in colon content among the treatment groups is shown in Fig. 4.4 A-J. The Bray-Curtis and Jaccard distances showed significant separation and clustering for NC, VA, and VAIL compared to PC ($P < 0.05$; Fig. 4.4 A, B, C, F, G, H), which is suggestive of the differences in colon microbiota composition among pigs fed these diets. In comparison with NC, VA and VAIL groups showed no clear clustering ($P > 0.05$; Fig 4.4 D, E, I, J).

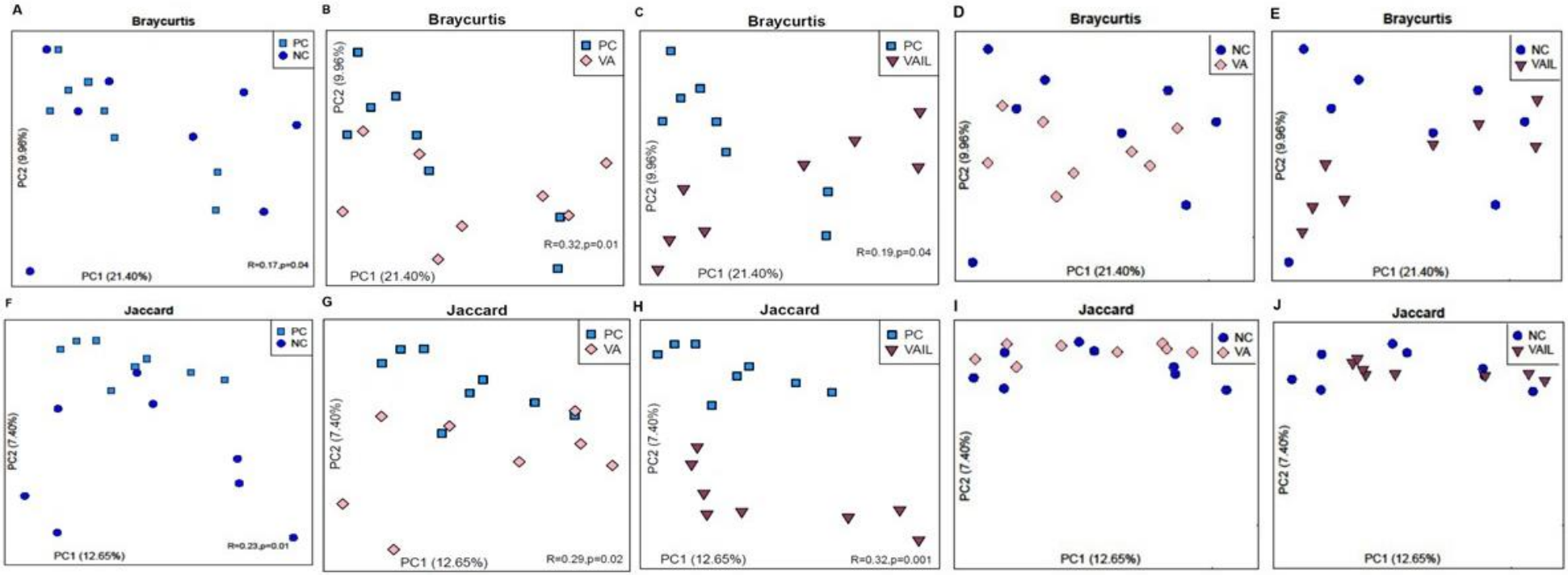


Figure 4.4. Beta diversity of colon bacterial community in pigs fed with very low protein diets supplemented with isoleucine (Ile) at NRC and valine (Val) above NRC levels, or a combination of the two.

Principal coordinates analysis (PCoA) based on (A-E) Bray-Curtis and (F-J) Jaccard. (A & F) NC vs. PC, (B & G) VA vs. PC, (C & H) VAIL vs. PC, (D & I) VA vs. NC; (E & J) VAIL vs. NC. ANOSIM was used to test the difference of beta diversity between diet treatments. PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (*i.e.*, lysine, methionine,

threonine, and tryptophan) at NRC (2012) levels; VA: NC containing Val above NRC level; IL: NC containing Ile at NRC level; VAIL: NC containing Val above NRC and Ile at NRC levels. Each node represents an individual pig. $n = 8$

Overall, at phylum level, Bacteroidetes, Actinobacteria and Spirochaetes and at genus level *Prevotella*, *Lactobacillus*, and *Megasphaera* were the most abundant bacteria in all diets (Fig. 4.5 A and B). The relative abundant of colon bacterial composition for individual pigs is shown in Supplementary Figure 4A.4.

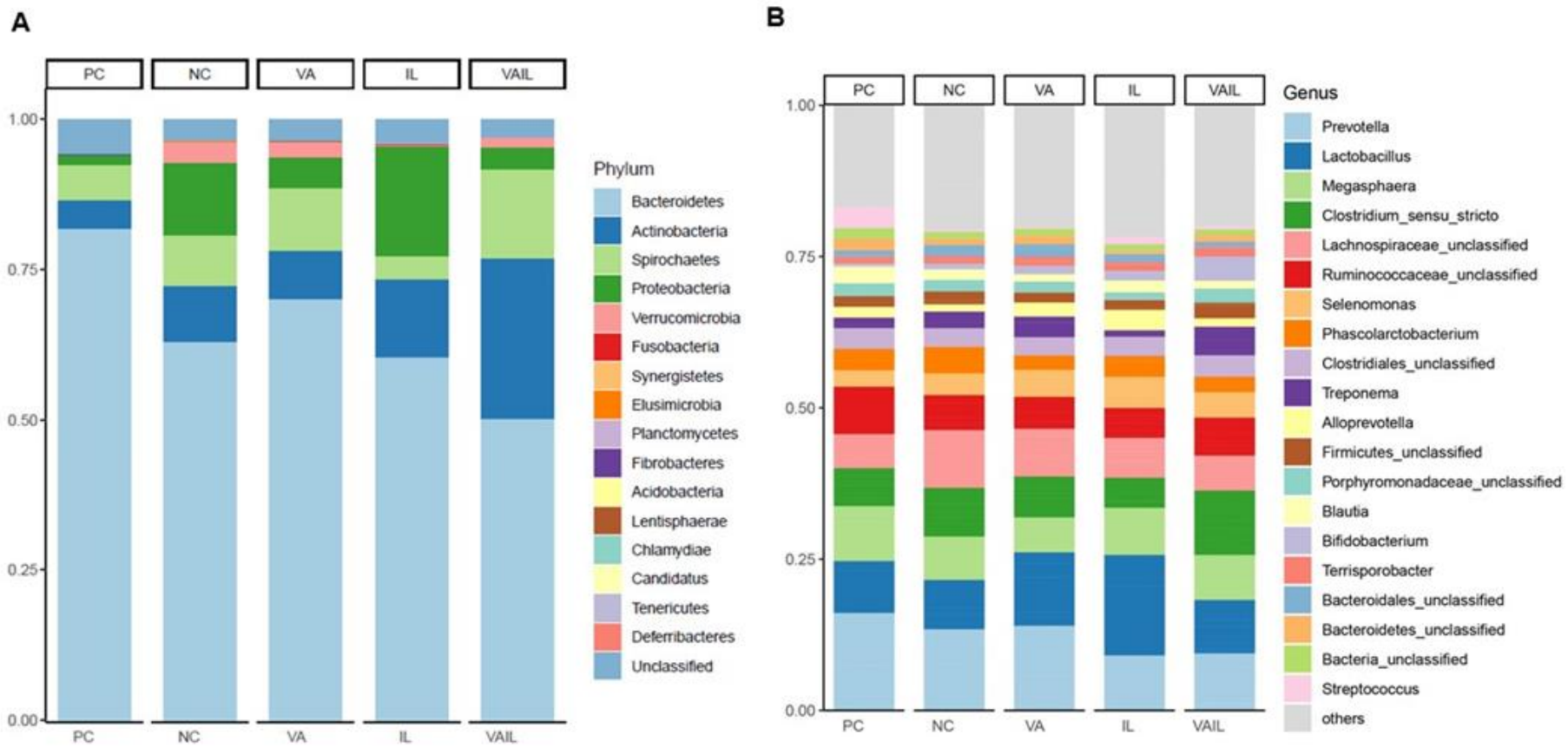
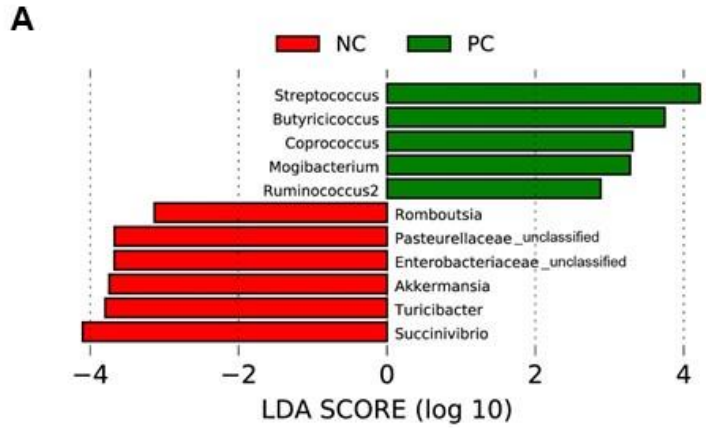
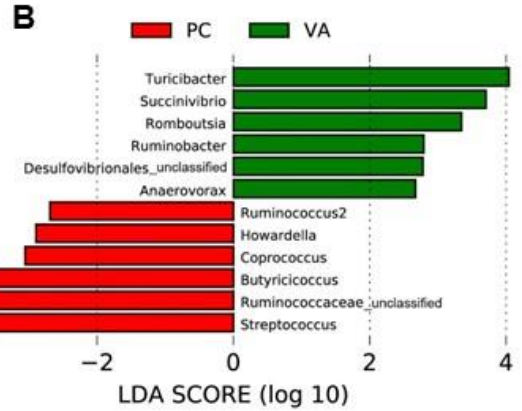


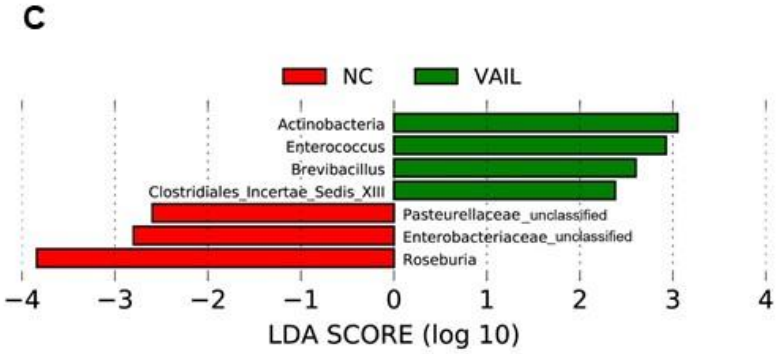
Figure 4.5. The relative abundance of colon bacterial composition at (A) phylum, and (B) genus levels in pigs fed with very low protein diets supplemented with isoleucine (Ile) at NRC and valine (Val) above NRC levels, or a combination of the two.

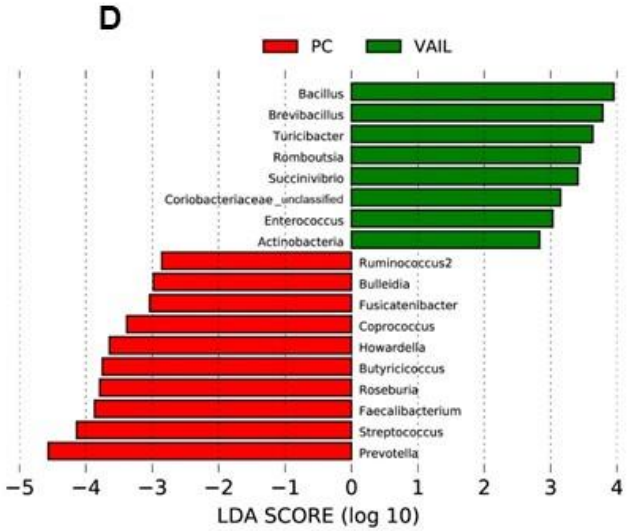
PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (*i.e.*, lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; VA: NC containing Val above NRC level; IL: NC containing Ile at NRC level; VAIL: NC containing Val above NRC and Ile at NRC levels. $n = 8$.

LDA with LefSe for bacterial communities between dietary groups are shown in Fig. 4.6. In comparison with PC, pigs fed with NC had higher proportions of *Succinivibrio*, *Turicibacter*, *Akkermansia*, *Enterobacteriaceae*, *Pasteurellaceae,* and *Romboutsia* (Fig. 4.6 A). Further pigs fed with VA had higher abundance of *Turicibacter*, *Succinivibrio*, *Romboutsia*, *Ruminobacter*, *Desulfovibrionales* and *Anaerovorax* relative to PC (Fig. 4.6B). VAIL had higher abundance of *Bacillus*, *Brevibacillus*, *Turicibacter*, *Romboutsia*, *Succinivibrio*, *Coriobacteriaceae*, *Enterococcus* and *Actinobacteria* in comparison with PC (Fig. 4.6 D). Relative to NC, pigs fed with VAIL had a higher abundance of *Actinobacteria, Enterococcus, Brevibacillus* and *Clostridiales_Incertae_ Sedis_ XIII* (Fig. 4.6 C). In comparison with NC, pigs fed with VA had higher abundance of *Mogibacterium* (Fig. 4.6 E).


A
NC
PC
Streptococcus
Butyricicoccus
Coprococcus
Mogibacterium
Ruminococcus2
Romboutsia
Pasteurellaceae_unclassified
Enterobacteriaceae_unclassified
Akkermansia
Turicibacter
Succinivibrio
−4
−2
0
2
4
LDA SCORE (log 10)


B
PC
VA
Turicibacter
Succinivibrio
Romboutsia
Ruminobacter
Desulfovibrionales_unclassified
Anaerovorax
Ruminococcus2
Howardella
Coprococcus
Butyricicoccus
Ruminococcaceae_unclassified
Streptococcus
−4
−2
0
2
4
LDA SCORE (log 10)


C
NC
VAIL
Actinobacteria
Enterococcus
Brevibacillus
Clostridiales_Incertae_Sedis_XIII
Pasteurellaceae_unclassified
Enterobacteriaceae_unclassified
Roseburia
−4
−3
−2
−1
0
1
2
3
4
LDA SCORE (log 10)


D
PC
VAIL
Bacillus
Brevibacillus
Turicibacter
Romboutsia
Succinivibrio
Coriobacteriaceae_unclassified
Enterococcus
Actinobacteria
Ruminococcus2
Bulleidia
Fusicatenibacter
Coprococcus
Howardella
Butyricicoccus
Roseburia
Faecalibacterium
Streptococcus
Prevotella
−5
−4
−3
−2
−1
0
1
2
3
4
LDA SCORE (log 10)

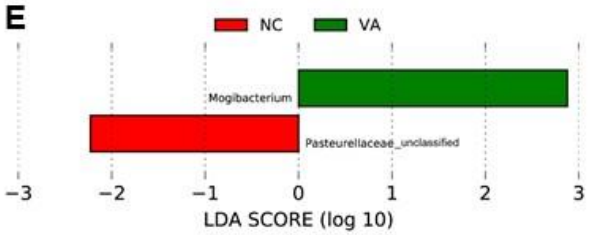

E
NC
VA
Mogibacterium
Pasteurellaceae_unclassified
−3
−2
−1
0
1
2
3
LDA SCORE (log 10)

Figure 4.6. Histograms of colon microbiota composition in pigs fed with very low protein diets supplemented with isoleucine (Ile) at NRC and valine (Val) above NRC levels, or a combination of the two using linear discriminant analysis (LDA) with effect size (LEfSe).

(A) NC vs. PC, (B) VA vs. PC, (C) VAIL vs. NC, (D) VAIL vs. PC, (E) VA vs. NC. PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (*i.e*., lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; VA: NC containing Val above NRC level; IL: NC containing Ile at NRC level; VAIL: NC containing Val above NRC and Ile at NRC levels. $n = 8$.

4.5. Discussion

Very low protein diets reduce the total N excretion [8–10], but they have an adverse effect on growth performance of pigs even when supplemented with FFL [11–14]. We and other have shown that supplementing BCAA, Val and a combination of Val and Ile at NRC levels partially improve the growth performance of pigs fed with VLP diets [16,18,24,28–33] 2418282930313233]. Little is understood whether a combination of Val above NRC levels and standard amount of Ile will have additive effects on growth of pigs offered with VLP diets and the mechanisms involved. Therefore, the objective of this study was to investigate the effect of Val above and Ile at NRC (2012) [15] levels on growth measurements, nutrient digestibility and gut microbiota in pigs fed with VLP diets. This study revealed several significant findings including: 1) supplementation of a combination of Val above and Ile at NRC (2012) [15] levels (VAIL) partially restored the adverse effect of VLP diets on growth (*e.g.*, BW, ADG); 2) VAIL and VA (VLP supplemented with Val above NRC levels) fully recovered the negative effects of VLP diets on ADFI; 3) VAIL and VA increased the thermal radiation and reduced the AFD of N which might be due to AA imbalance that contribute to partial or lack of complete recovery of growth in these groups; 4) the colon content of VAIL group had higher abundance of *Actinobacteria, Enterococcus*, and *Brevibacillus* and that of VA was more enriched with *Mogibacterium* while pigs fed with VLP diets (NC) had higher *Pasteurellaceae* and *Enterobacteriaceae*, which all may contribute to observed growth efficiency in above mentioned groups. Overall VAIL improved the growth performance of pigs fed with VLP diets likely through changing the composition of gut microbiota.

In line with previous studies [11–14] reduction in dietary CP more than 4% while supplementing FFL had an adverse effect on feed intake and growth performance of pigs. While VA and VAIL completely recovered the feed intake of pigs fed with VLP diets, these groups partially improved the growth, which is in line with previous research showing that supplementation of Val and a combination of Val and Ile into VLP diets containing FFL promotes growth and feed intake in pigs

[18,28–33]. We and others have previously shown that supplementation of VLP or SLP diets with BCAA or Val increased the transcript of orexigenic neuropeptide Y and agouti-related protein and decreased the anorexigenic proopiomelanocortin, melanocortin-4-receptor and cocaine- and amphetamine regulated transcript in hypothalamus, which may contribute to improved feed intake in BCAA supplemented groups [17,26,38,39]. We recently showed that Val and Ile combination added at NRC (2012) [15] levels into VLP diets improved the growth performance to the levels seen in pigs received standard protein diet [24]. The partial, but not complete recovery in growth performance in VA and VAIL groups in this study might be due to AA imbalances resulted from higher ratio of Val:Ile or ratio of Val to other AA. Excess dietary Val causing AA imbalances seems to interfere with the supply of Ile. The higher level of dietary Val in VAIL and VA groups may increase the α-ketoisovalerate, a keto acid produced by degradation of Val, levels that can allosterically inhibit the branched-chain α-ketoacid dehydrogenase which is involved in the first step of all three BCAA catabolism [51]. Therefore, excess dietary Val may result in reduction of plasma concentration of Ile as reported previously [52].

The AA imbalances caused by higher ratio of Val to Ile or other AA in pigs fed with VA and VAIL groups may also deteriorate the growth performance through influencing their energy and nutrients retention. In the current study, VA and VAIL groups increased the thermal radiation. This data is consistent with our previous study [24] that supplementation of VLP diet with combination of Val and Ile at NRC levels stimulated the energy loss and thermal radiation. The AA imbalances as result of higher Val to other AA ratio is likely detected by sensors with the subsequent activation or inhibition of downstream pathways resulting in increased energy expenditure [53]. Due to the fact that some AA share the same transport system, it remains to be determined whether reduction of Leu and Ile or other AA due to higher level of dietary Val contribute to increased energy loss in VA and VAIL groups. In the present study, the AFD of N was reduced in VA and VAIL groups. Other studies showed that adding Val alone or in combination with Ile had either no effect on [2]

or increased [54] N retention in pigs fed with SLP diet. The discrepancy in results of above-mentioned studies and the current study might be explained by different diets crude protein content and the ratio of Val to other AA. Since Val was added at a greater level than NRC values in this study, the reduced AFD of N in the VA and VAIL groups might be linked to AA imbalances. It has been previously reported that AA imbalances can cause growth retardation and reduce the limiting AA and N utilization efficiency [55].

Previous studies have suggested several mechanisms for the positive effects of BCAA on growth performance of pigs such as improving the gut development [24,34–36], feed intake [17,26,37–39], nutrients and AA utilization [40–42, insulin-like growth factor 1 (IGF-1) signaling [17], and muscle protein synthesis [26,44–46]. Further, we and others showed that the stimulatory effects of BCAA on growth may be associated with alteration in gut microbiota composition [43,16]. It is unclear whether partial improvement in growth in VA and VAIL groups in the current study is associated with changes in gut microbiota. In our study, Bacteroidetes, Actinobacteria and Spirochaetes at phylum level, and *Prevotella*, *Lactobacillus*, and *Megasphaera* at genus level were the three most abundant bacteria in the colon contents of all dietary groups. Similarly, we and others previously reported that Bacteroidetes, Actinobacteria and Spirochaetes together with Firmicutes were among the main phyla in the feces, cecal digesta and colonic contents of pigs [13,16,47,56–59]. Further, in line with our data others showed that the abundance of *Prevotella* in the gut is increased after weaning, which is likely due to their increased ability in digestion of plant-based diets containing hemicelluloses and xylans [13,57,60–62]. Likewise, *Lactobacillus and Megasphaera* have both been shown to play a role in carbohydrate fermentation [63,64]. Other studies have reported bacterial communities such as *Streptococcus* and *Lactobacillus* as most abundant communities in the gut microbiome of pigs [65,66]. These differences in the main populations of gut microbiome might be related to differences in pigs breed, age, feed, and husbandry. In the current study, the

Simpson index for bacterial populations in the colon was different in VAIL when compared to PC and VA which is suggestive of differences in species diversity in these groups.

In this study, the relative abundance of *Pasteurellaceae*, and *Enterobacteriaceae* was higher in pigs fed with VLP diets. Similarly, others showed an increase in abundance of *Enterobacteriaceae* in colon of weaned pigs fed with low protein diets [67]. *Pasteurellaceae*, and some members of *Enterobacteriaceae* are pathogenic bacteria [68,69]. In a previous study an increase in abundance of *Enterobacteriaceae* in diarrhoeic pigs was shown during post-weaning period [70]. The abundance of *Enterobacteriaceae* and intestinal chronic inflammatory diseases have been reported to be positively correlated [71]. Opportunistic pathogens such as *Salmonella Typhimurium* and *enterotoxigenic Escherichia coli* are members of the *Enterobacteriaceae* family that can cause intestinal inflammation post-weaning diarrhea in piglets [72–74]. These results suggest that pigs fed with VLP reduce the growth performance likely due to gastrointestinal disturbances related with increased abundance of pathogenic bacteria that are potentially involved in gut inflammation. Further research is required to determine whether adverse effects of VLP diets on growth performance is associated with changes in certain inflammatory markers in post weaned pigs.

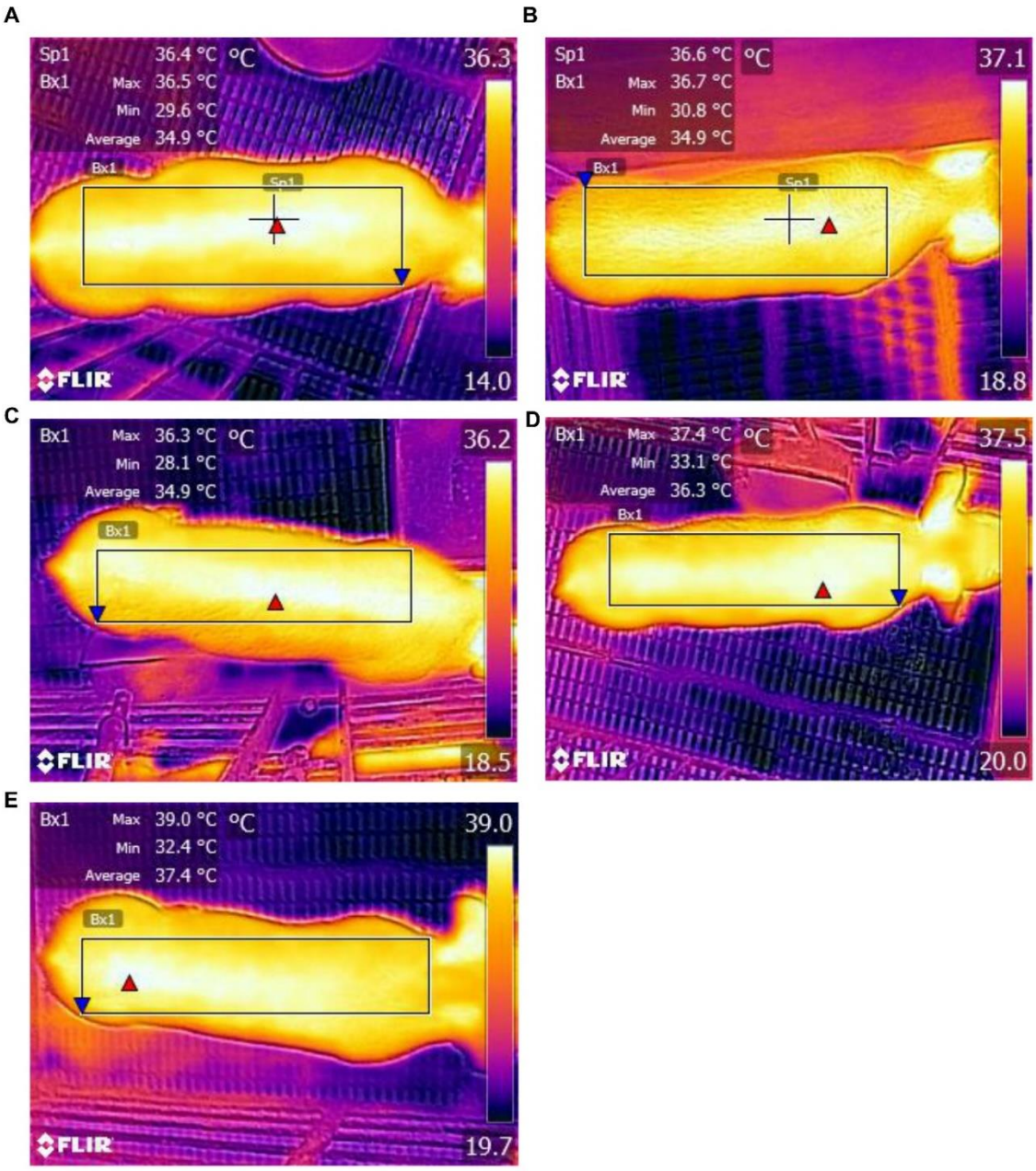
The colon content of pigs offered with VAIL had higher abundance of *Actinobacteria*, *Enterococcus*, and *Brevibacillus* and that of VA was more enriched with *Mogibacterium*. *Actinobacteria* has been the focus of many studies due their importance for the maintenance of gut homeostasis as well as their potential for therapeutic use for gastrointestinal pathological conditions and systemic diseases [75]. The abundance of *Actinobacteria* has been reported to be decreased in patients with coeliac disease [76] or irritable bowel syndrome [77]. Therefore, *Actinobacteria* are thought to play an important role in health [78]. *Enterococcus faecium* has been used as a probiotic supplement in pigs' diet with beneficial effects on reducing diarrhea and pathogenic *E. coli* in the intestine and improving growth performance and microbiota composition [79–81]. It appears that *Enterococci* improve growth performance either through producing organic acids that results in

lowering pH and suppressing the pathogen strains in the gut [82], or inhibiting the pathogen's adhesion to the intestinal mucosa [80]. *Brevibacillus brevis* is a unique candidate with broad-spectrum antimicrobial action [83] and has the potential to improve the growth performance and gut microbiota composition in pigs [84]. Previous studies have reported a positive link between the abundance of *Mogibacterium* and short-chain fatty acids concentration in feces of weaned pigs suggestive of a possible health benefit in these animals [85]. Overall, these data indicate that the improvement in growth performance of pigs fed with VAIL and VA might be due to probiotic and antibacterial properties and health benefits of their highly abundant colonic bacteria, *i.e., Actinobacteria*, *Enterococcus*, *Brevibacillus* and *Mogibacterium.*

4.6. Conclusions

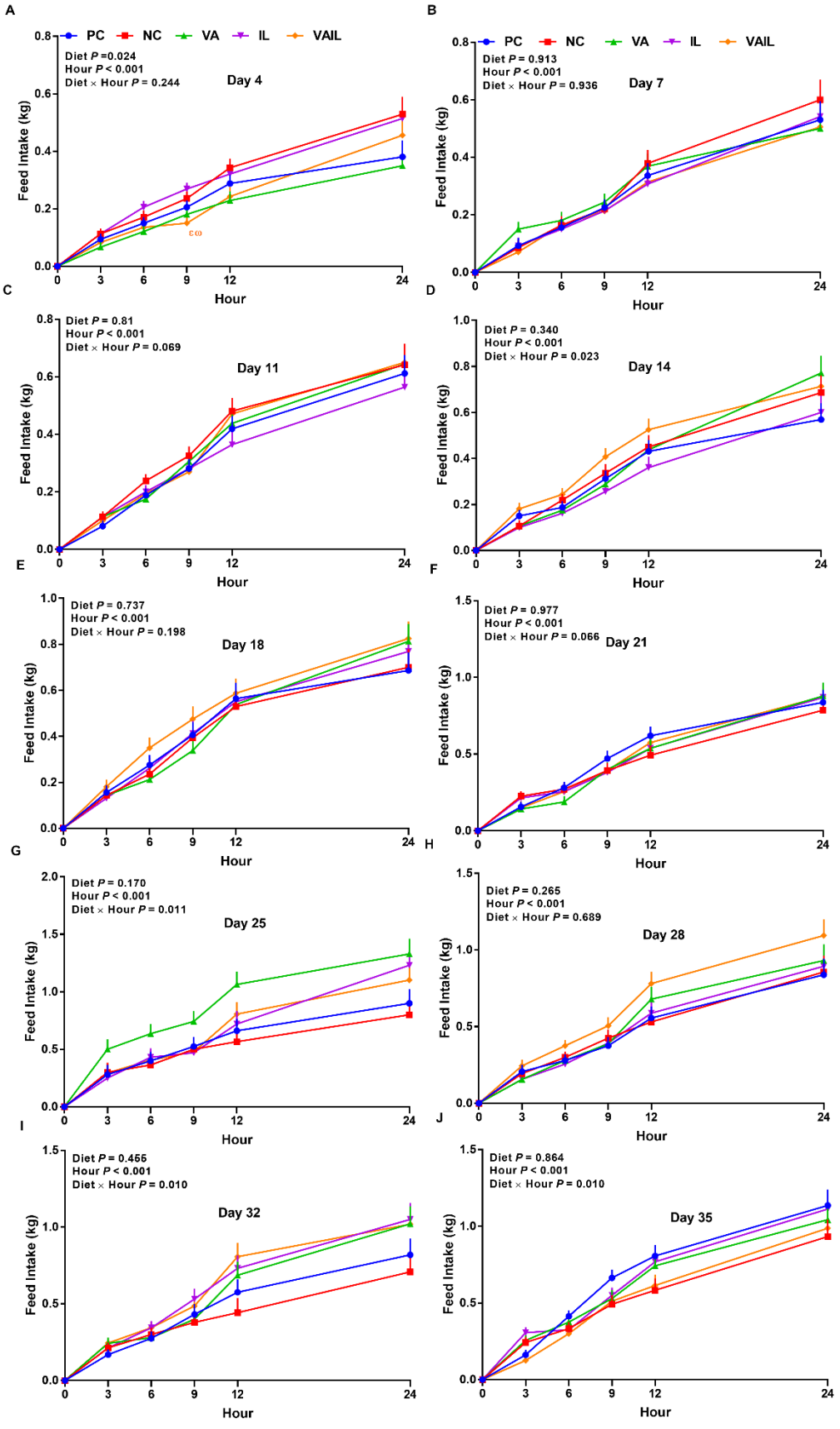
In this study, for the first time, the effect of Val above and Ile at NRC levels on growth performance, nutrient digestibility, and gut microbiota in pigs fed VLP diets was addressed. We demonstrate that supplementing VLP diet with a mixture of Ile and added Val, i.e., VAIL, fully recovered the feed intake while partially restored the growth performance of pigs likely through increasing the abundance of *Actinobacteria*, *Enterococcus*, and *Brevibacillus* in colon. The partial, but not complete recovery in growth performance in VAIL group is likely associated with AA imbalances causing increased thermal radiation and reduced AFD of N in this group.

4.7. Appendices



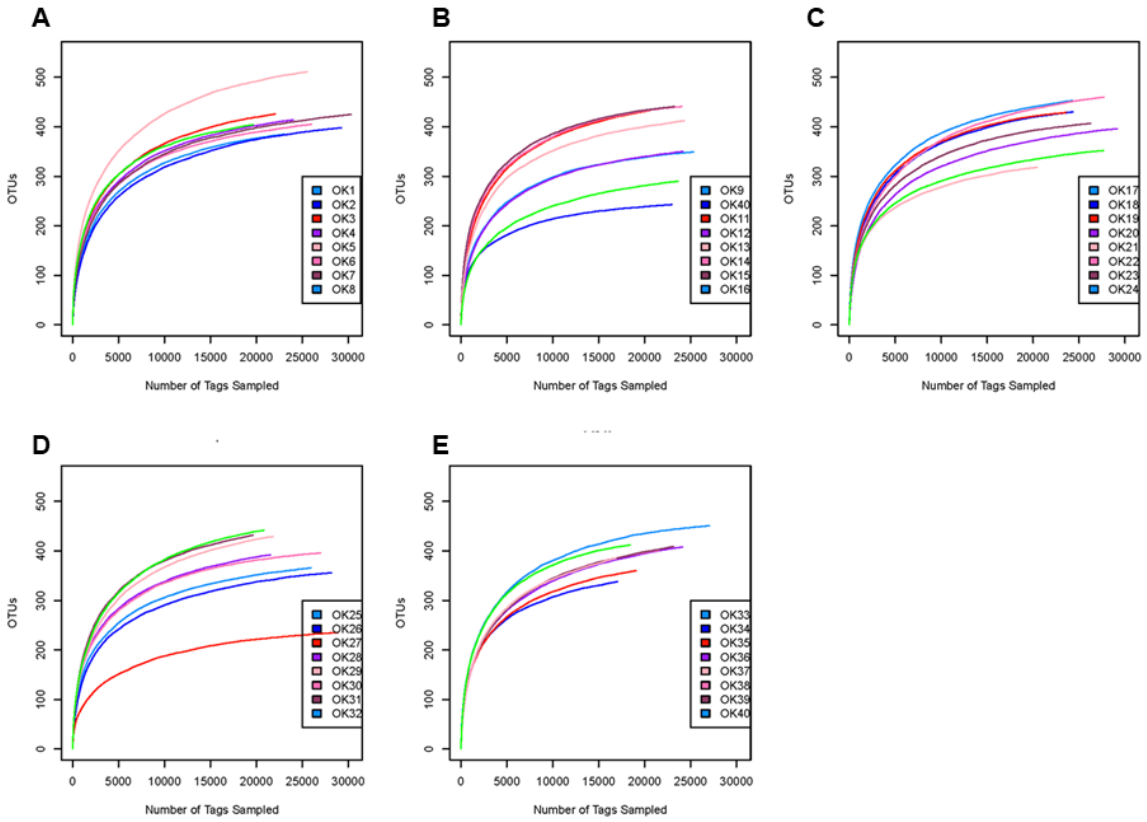
Appendix Figure 4A.1. Representative thermal images of nursery pigs fed with very low protein diets supplemented with isoleucine (Ile) at NRC and valine (Val) above NRC levels, or a combination of the two.

FLIR camera software (FLIR Research Studio, Wilsonville, OR) was used to calculate the mean of dorsal body surface temperature by drawing a rectangle from shoulder to rump. (A) PC, (B) NC, (C) VA, (D) IL, and (E) VAIL. PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (i.e., lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; VA: NC containing Val above NRC level; IL: NC containing Ile at NRC level; VAIL: NC containing Val above NRC and Ile at NRC levels.


A
PC NC VA IL VAIL
Diet P =0.024
Hour P < 0.001
Diet × Hour P = 0.244
Day 4
Feed Intake (kg)
Hour
B
Diet P = 0.913
Hour P < 0.001
Diet × Hour P = 0.936
Day 7
C
Diet P = 0.81
Hour P < 0.001
Diet × Hour P = 0.069
Day 11
D
Diet P = 0.340
Hour P < 0.001
Diet × Hour P = 0.023
Day 14
E
Diet P = 0.737
Hour P < 0.001
Diet × Hour P = 0.198
Day 18
F
Diet P = 0.977
Hour P < 0.001
Diet × Hour P = 0.066
Day 21
G
Diet P = 0.170
Hour P < 0.001
Diet × Hour P = 0.011
Day 25
H
Diet P = 0.265
Hour P < 0.001
Diet × Hour P = 0.689
Day 28
I
Diet P = 0.455
Hour P < 0.001
Diet × Hour P = 0.010
Day 32
J
Diet P = 0.864
Hour P < 0.001
Diet × Hour P = 0.010
Day 35

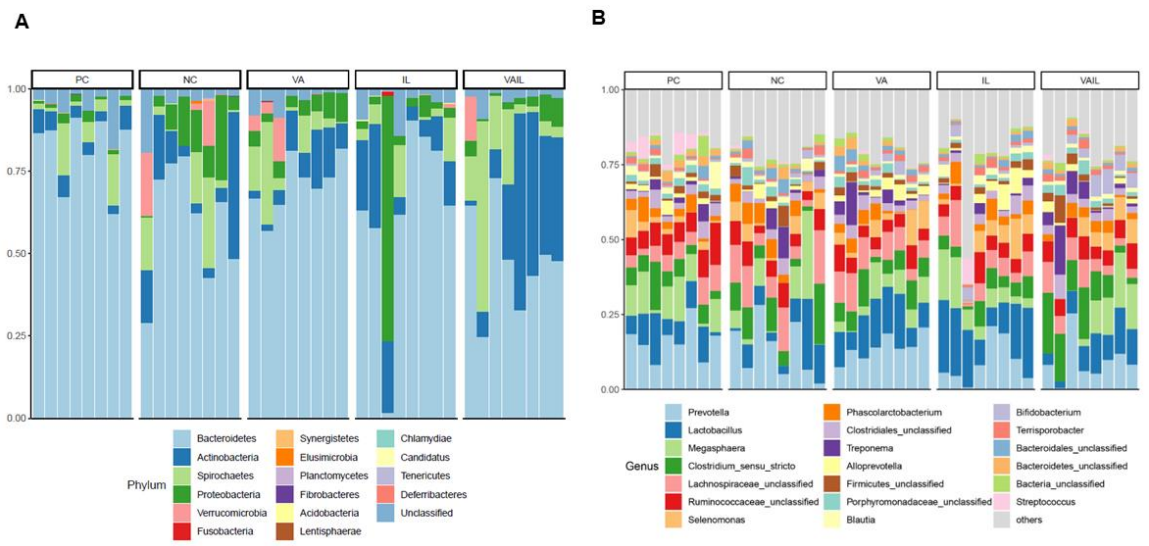
Appendix Figure 4A.2. Feed intake at day (A) 4, (B) 7, (C) 11 (D) 14, (E) 18, (F) 21, (G) 25, (H) 28, (I) 32, and (J) 35 in nursery pigs fed with very low protein diets supplemented with isoleucine (Ile) at NRC and valine (Val) above NRC levels, or a combination of the two.

PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (i.e., lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; VA: NC containing Val above NRC level; IL: NC containing Ile at NRC level; VAIL: NC containing Val above NRC and Ile at NRC levels. ω $P \leq 0.1$ VAIL vs. NC, ε $P \leq 0.1$ VAIL vs. PC. The values are means ± standard error of the mean. n=8.



Appendix Figure 4A.3. Rarefaction curve analysis for colon samples collected from nursery pigs fed with very low protein diets supplemented with isoleucine (Ile) at NRC and valine (Val) above NRC levels, or a combination of the two.

The rarefaction curves illustrate the number of operational taxonomic units (OTUs) discovered as a function of the number of reads sampled when data were evaluated for individual animals. (A) PC, (B) NC, (C) VA, (D) IL, and (E) VAIL PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (i.e., lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; VA: NC containing Val above NRC level; IL: NC containing Ile at NRC level; VAIL: NC containing Val above NRC and Ile at NRC levels. n = 8.



Appendix Figure 4A.4. The relative abundance of colon bacterial composition at (A) phylum, and (B) genus levels for individual pigs fed with very low protein diets supplemented with isoleucine (Ile) at NRC and valine (Val) above NRC levels, or a combination of the two.

PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (i.e., lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; VA: NC containing Val above NRC level; IL: NC containing Ile at NRC level; VAIL: NC containing Val above NRC and Ile at NRC levels. n = 8 for each dietary group.

4.8. References

1. Wang, Y.; Zhou, J.; Wang, G.; Cai, S.; Zeng, X.; Qiao, S. Advances in low-protein diets for swine. *J. Anim. Sci. Biotechnol.* **2018**, *9*, 1–14. DOI: 10.1186/s40104-018-0276-7

2. Lordelo, M.; Gaspar, A.; Le Bellego, L.; Freire, J. Isoleucine and valine supplementation of a low-protein corn-wheat-soybean meal-based diet for piglets: Growth performance and nitrogen balance. *J. Anim. Sci.* **2008**, *86*, 2936–2941. DOI: 10.2527/jas.2007-0222

3. Opapeju, F.; Rademacher, M.; Blank, G.; Nyachoti, C. Effect of low-protein amino acid-supplemented diets on the growth performance, gut morphology, organ weights and digesta characteristics of weaned pigs. *Animal* **2008**, *2*, 1457–1464. DOI: 10.1017/S175173110800270X

4. Le Bellego, L.; van Milgen, J.; Noblet, J. Effect of high temperature and low-protein diets on the performance of growing-finishing pigs. *J. Anim. Sci.* **2002**, *80*, 691–701. DOI: 10.2527/2002.803691x

5. Shriver, J.A.; Carter, S.D.; Sutton, A.L.; Richert, B.T.; Senne, B.W.; Pettey, L.A. Effects of adding fiber sources to reduced-crude protein, amino acid-supplemented diets on nitrogen excretion, growth performance, and carcass traits of finishing pigs. *J. Anim. Sci.* **2003**, *81*, 492–502. DOI: 10.2527/2003.812492x

6. Madrid, J.; Martínez, S.; López, C.; Orengo, J.; López, M.J.; Hernández, F. Effects of low protein diets on growth performance, carcass traits and ammonia emission of barrows and gilts. *Anim. Prod. Sci.* **2012**, *53*, 146–153. DOI: 10.1071/AN12067

7. Figueroa, J.; Lewis, A.; Miller, P.S.; Fischer, R.; Gómez, R.; Diedrichsen, R. Nitrogen metabolism and growth performance of gilts fed standard corn-soybean meal diets or low-crude protein, amino acid-supplemented diets. *J. Anim. Sci.* **2002**, *80*, 2911–2919. DOI: 10.2527/2002.80112911x

8. Deng, D.; Huang, R.L.; Li, T.J.; Wu, G.Y.; Xie, M.Y.; Tang, Z.R.; Kang, P.; Zhang, Y.M.; Fan, M.Z.; Kong, X.F.; Ruan, Z. Nitrogen balance in barrows fed low-protein diets supplemented with essential amino acids. *Livest. Sci.* **2007**, *109*, 220–223. DOI: 10.1016/j.livsci.2007.01.122

9. He, L.; Wu, L.; Xu, Z.; Li, T.; Yao, K.; Cui, Z.; Yin, Y.; Wu, G. Low-protein diets affect ileal amino acid digestibility and gene expression of digestive enzymes in growing and finishing pigs. *Amino acids* **2016**, *48*, 21–30. DOI: 10.1007/s00726-015-2059-1

10. Portejoie, S.; Dourmad, J.Y.; Martinez, J.; Lebreton, Y. Effect of lowering dietary crude protein on nitrogen excretion, manure composition and ammonia emission from fattening pigs. *Livest. Prod. Sci.* **2004**, *91*, 45–55. DOI: 10.1016/j.livprodsci.2004.06.013

11. Nyachoti, C.M.; Omogbenigun, F.O.; Rademacher, M.; Blank, G. Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. *J. Anim. Sci.* **2006**, *84*, 125–134. DOI: 10.2527/2006.841125x

12. Wu, L.; Liao, P.; He, Q.; Tan, B.; Guo, F.; Tang, M.; Li, T. Chronic feeding with protein-restricted diets affect ileal amino acid digestibility and the expression of nutrient-sensing, hormone secretion, gastrointestinal digestive enzyme, and nutrient transporter genes in young weaned pigs. *Oncotarget* **2018**, *5*, 1–15. Retrieved from https://www.oncotarget.com/article/24093/text/

13. Spring, S.; Premathilake, H.; DeSilva, U.; Shili, C.; Carter, S.; Pezeshki, A. Low protein-high carbohydrate diets alter energy balance, gut microbiota composition and blood metabolomics profile in young pigs. *Sci. Rep.* **2020**, *10*, 3318. DOI: 10.1038/s41598-020-60150-y

14. Luo, Z.; Li, C.; Cheng, Y.; Hang, S.; Zhu, W. Effects of low dietary protein on the metabolites and microbial communities in the caecal digesta of piglets. *Arch. Anim. Nutr.* **2015**, *69*, 212–226. DOI: 10.1080/1745039X.2015.1034521

15. NRC. *Nutrient Requirements of Swine: Eleventh Revised Edition*; The National Academies Press: Washington, DC, USA, 2012.

16. Spring, S.; Premathilake, H.; Bradway, C.; Shili, C.; DeSilva, U.; Carter, S.; Pezeshki, A. Effect of very low-protein diets supplemented with branched-chain amino acids on energy balance, plasma metabolomics and fecal microbiome of pigs. *Sci. Rep.* **2020**, *10*, 15859. DOI: 10.1038/s41598-020-72816-8

17. Habibi, M.; Shili, C.; Sutton, J.; Goodarzi, P.; Maylem, E.R.; Spicer, L.; Pezeshki, A. Branched-chain amino acids partially recover the reduced growth of pigs fed with protein-restricted diets through both central and peripheral factors. *Anim Nutr.* **2021**, *7*, 868–882. DOI: 10.1016/j.aninu.2021.02.002

18. Figueroa, J.; Lewis, A.; Miller, P.S.; Fischer, R.; Diedrichsen, R. Growth, carcass traits, and plasma amino acid concentrations of gilts fed low-protein diets supplemented with amino acids including histidine, isoleucine, and valine. *J. Anim. Sci.* **2003**, *81*, 1529–1537. DOI: 10.2527/2003.8161529x

19. Zhao, Y.; Tian, G.; Chen, D.; Zheng, P.; Yu, J.; He, J.; Mao, X.; Huang, Z.; Luo, Y.; Luo, J.; Yu, B. Effect of different dietary protein levels and amino acids supplementation patterns on growth performance, carcass characteristics and nitrogen excretion in growing-finishing pigs. *J. Anim. Sci. Biotechnol.* **2019**, *10*, 1–10. DOI: 10.1186/s40104-019-0381-2

20. Gloaguen, M.; Le Floc'H, N.; Brossard, L.; Barea, R.; Primot, Y.; Corrent, E.; van Milgen, J. Response of piglets to the valine content in diet in combination with the supply of other branched-chain amino acids. *Animal.* **2011**, *5*, 1734–1742. DOI: 10.1017/S1751731111000760

21. Gloaguen, M.; Le Floc'H, N.; Corrent, E.; Primot, Y.; van Milgen, J. Providing a diet deficient in valine but with excess leucine results in a rapid decrease in feed intake and modifies the

postprandial plasma amino acid and α-keto acid concentrations in pigs. *J. Anim. Sci.* **2012**, *90*, 3135–3142. DOI: 10.2527/jas.2011-4956

22. Wiltafsky, M.K.; Pfaffl, M.W.; Roth, F.X. The effects of branched-chain amino acid interactions on growth performance, blood metabolites, enzyme kinetics and transcriptomics in weaned pigs. *Br. J. Nutr.* **2010**, *103*, 964–976. DOI: 10.1017/S0007114509992212

23. Edmonds, M.; Baker, D. Amino acid excesses for young pigs: Effects of excess methionine, tryptophan, threonine or leucine. *J. Anim. Sci.* **1987**, *64*, 1664–1671. DOI: 10.2527/jas1987.6461664x

24. Habibi, M.; Goodarzi, P.; Shili, C.N.; Sutton, J.; Wileman, C.M.; Kim, D.M.; Lin, D.; Pezeshki, A. A Mixture of Valine and Isoleucine Restores the Growth of Protein-Restricted Pigs Likely through Improved Gut Development, Hepatic IGF-1 Pathway, and Plasma Metabolomic Profile. *Int J Mol Sci.* **2022**, *23*, 3300. DOI: 10.3390/ijms23063300

25. Nørgaard, J.V.; Fernández, J.A. Isoleucine and valine supplementation of crude protein-reduced diets for pigs aged 5–8 weeks. *Anim. Feed Sci. Technol.* **2009**, *154*, 248–253. DOI: 10.1016/j.anifeedsci.2009.08.010

26. Zhang, X.; Liu, X.; Jia, H.; He, P.; Mao, X.; Qiao, S.; Zeng, X. Valine supplementation in a reduced protein diet regulates growth performance partially through modulation of plasma amino acids profile, metabolic responses, endocrine, and neural factors in piglets. *J. Agric. Food Chem.* **2018**, *66*, 3161–3168. DOI: 10.1021/acs.jafc.8b01113

27. Cheng, L.; Jin, Y.H.; Kim, I.H. Effects of Bacillus licheniformis derived-protease supplementation, alone or in combination, with valine to low protein diet on growth performance and carcass quality grade in growing-finishing pigs. *J. Appl. Anim. Res.* **2021**, *49*, 180–184. DOI: 10.1080/09712119.2021.1933494

28. Ma, W.; Mao, P.; Fan, W.; Zhu, Y.; Guo, L. Valine and isoleucine supplementation improve performance and serum biochemical concentrations in growing gilts fed low-protein diets. *Can. J. Anim. Sci.* **2019**, *99*, 921–928. DOI: 10.1139/cjas-2018-0169

29. Russell, L.E.; Kerr, B.J.; Easter, R.A. Limiting amino acids in an 11% crude protein corn-soybean meal diet for growing pigs. *J. Anim. Sci.* **1987**, *65*, 1266–1272. DOI: 10.2527/jas1987.6551266x

30. Mavromichalis, I.; Webel, D.M.; Emmert, J.L.; Moser, R.L.; Baker, D.H. Limiting order of amino acids in a low-protein corn-soybean meal-whey-based diet for nursery pigs. *J. Anim. Sci.* **1998**, *76*, 2833–2837. DOI: 10.2527/1998.76112833x

31. Liu, X.; Ma, W.; Zeng, X.; Xie, C.; Thacker, P.; Htoo, J.; Qiao, S.Y. Estimation of the standardized ileal digestible valine to lysine ratio required for 25-to 120-kg pigs fed low crude protein diets supplemented with crystalline amino acids. *J. Anim. Sci.* **2015**, *93*, 4761–4773. DOI: 10.2527/jas.2015-9308

32. Waguespack, A.; Bidner, T.; Payne, R.; Southern, L. Valine and isoleucine requirement of 20-to 45-kg pigs. *J. Anim. Sci.* **2012**, *90*, 2276–2284. DOI: 10.2527/jas.2011-4454

33. Powell, S.; Bidner, T.; Payne, R.; Southern, L. Growth performance of 20-to 50-kg pigs fed low-crude-protein diets supplemented with histidine, cystine, glycine, glutamic acid, or arginine. *J. Anim. Sci.* **2011**, *89*, 3643–3650. DOI: 10.2527/jas.2010-3757

34. Ren, M.; Zhang, S.; Zeng, X.; Liu, H.; Qiao, S. Branched-chain amino acids are beneficial to maintain growth performance and intestinal immune-related function in weaned piglets fed protein restricted diet. *Asian-Australas. J. Anim. Sci.* **2015**, *28*, 1742. DOI: 10.5713/ajas.14.0131

35. Sun, Y.; Wu, Z.; Li, W.; Zhang, C.; Sun, K.; Ji, Y.; Wang, B.; Jiao, N.; He, B.; Wang, W.; et al. Dietary L-leucine supplementation enhances intestinal development in suckling piglets. *Amino Acids* **2015**, *47*, 1517–1525. DOI: 10.1007/s00726-015-1985-2

36. Duan, Y.; Tan, B.; Li, J.; Liao, P.; Huang, B.; Li, F.; Xiao, H.; Liu, Y.; Yin, Y. Optimal branched-chain amino acid ratio improves cell proliferation and protein metabolism of porcine enterocytesin vivo and in vitro. *Nutr.* **2018**, *54*, 173–181. DOI: 10.1016/j.nut.2018.03.057

37. Zhang, S.; Qiao, S.; Ren, M.; Zeng, X.; Ma, X.; Wu, Z.; Thacker, P.; Wu, G. Supplementation with branched-chain amino acids to a low-protein diet regulates intestinal expression of amino acid and peptide transporters in weanling pigs. *Amino Acids* **2013**, *45*, 1191–1205. DOI: 10.1007/s00726-013-1577-y

38. Zheng, L.; Wei, H.; Cheng, C.; Xiang, Q.; Pang, J.; Peng, J. Supplementation of branched-chain amino acids to a reduced-protein diet improves growth performance in piglets: Involvement of increased feed intake and direct muscle growth-promoting effect. *Br. J. Nutr.* **2016**, *115*, 2236–2245. DOI: 10.1017/S0007114516000842

39. Tian, M.; Heng, J.; Song, H.; Shi, K.; Lin, X.; Chen, F.; Guan, W.; Zhang, S. Dietary branched-chain amino acids regulate food intake partly through intestinal and hypothalamic amino acid receptors in piglets. *J. Agric. Food Chem.* **2019**, *67*, 6809–6818. DOI: 10.1021/acs.jafc.9b02381

40. Zhou, H.; Yu, B.; Gao, J.; Htoo, J.K.; Chen, D. Regulation of intestinal health by branched-chain amino acids. *Anim. Sci. J.* **2018**, *89*, 3–11. DOI: 10.1111/asj.12937

41. Habibi, M.; Shili, C.N.; Sutton, J.; Goodarzi, P.; Pezeshki, A. Dietary branched-chain amino acids modulate the dynamics of calcium absorption and reabsorption in protein-restricted pigs. *J. Anim. Sci. Biotechnol.* **2022**, *13*, 1–16. DOI: 10.1186/s40104-021-00669-w

42. Kwon, W.B.; Soto, J.A.; Stein, H.H. Effects on nitrogen balance and metabolism of branched-chain amino acids by growing pigs of supplementing isoleucine and valine to diets with adequate or excess concentrations of dietary leucine. *J. Anim. Sci.* **2020**, *98*, skaa346. DOI: 10.1093/jas/skaa346

43. Yin, J.; Ma, J.; Li, Y.; Ma, X.; Chen, J.; Zhang, H.; Wu, X.; Li, F.; Liu, Z.; Li, T.; Yin, Y. Branched-chain amino acids, especially of leucine and valine, mediate the protein restricted response in a piglet model. *Food Funct.* **2020**, *11*, 1304–1311. DOI: 10.1039/C9FO01757G

44. Suryawan, A.; Jeyapalan, A.S.; Orellana, R.A.; Wilson, F.A.; Nguyen, H.V.; Davis, T.A. Leucine stimulates protein synthesis in skeletal muscle of neonatal pigs by enhancing mTORC1 activation. *Am. J. Physiol. Endocrinol. Metab.* **2008**, *295*, 868–875. DOI: 10.1152/ajpendo.90314.2008

45. Murgas Torrazza, R.; Suryawan, A.; Gazzaneo, M.C.; Orellana, R.A.; Frank, J.W.; Nguyen, H.V.; Fiorotto, M.L.; El-Kadi, S.; Davis, T.A. Leucine supplementation of a low-protein meal increases skeletal muscle and visceral tissue protein synthesis in neonatal pigs by stimulating mTOR-dependent translation initiation. *J. Nutr.* **2010**, *140*, 2145–2152. DOI: 10.3945/jn.110.128421

46. Yin, Y.; Yao, K.; Liu, Z.; Gong, M.; Ruan, Z.; Deng, D.; Tan, B.; Liu, Z.; Wu, G. Supplementing L-leucine to a low-protein diet increases tissue protein synthesis in weanling pigs. *Amino Acids* **2010**, *39*, 1477–1486. DOI: 10.1007/s00726-010-0612-5

47. Shili, C.N.; Broomhead, J.N.; Spring, S.C.; Lanahan, M.B.; Pezeshki, A. A novel corn-expressed phytase improves daily weight gain, protein efficiency ratio and nutrients digestibility and alters fecal microbiota in pigs fed with very low protein diets. Animals 2020, 10, 1926. DOI: 10.3390/ani10101926

48. Goodarzi, P.; Habibi, M.; Roberts, K.; Sutton, J.; Shili, C.N.; Lin, D.; Pezeshki, A. Dietary tryptophan supplementation alters fat and glucose metabolism in a low-birthweight piglet model. *Nutrients* **2021**, *13*, 2561. DOI: 10.3390/nu13082561

49. Kozich, J.J.; Westcott, S.L.; Baxter, N.T.; Highlander, S.K.; Schloss, P.D. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq

Illumina sequencing platform. Applied and environmental microbiology. *Appl. Environ. Microbiol.* **2013**, *79*, 5112–5120. DOI: 0.1128/AEM.01043-13

50. Cole, J.R.; Wang, Q.; Cardenas, E.; Fish, J.; Chai, B.; Farris, R.J.; Kulam-Syed-Mohideen, A.S.; McGarrell, D.M.; Marsh, T.; Garrity, G.M.; Tiedje, J.M. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Res.* **2009**, *37*, 141–145. DOI: 10.1093/nar/gkn879

51. 51. Brosnan, J.T.; Brosnan, M.E. Branched-chain amino acids: enzyme and substrate regulation. *J Nutr.* **2006**, *136*, 207–211. DOI: 10.1093/jn/136.1.207S

52. Smith, T.K.; Austic, R.E. The branched-chain amino acid antagonism in chicks. *J. Nutr.* **1978**, *108*, 1180–1191. DOI: 10.1093/jn/108.7.1180

53. Xiao, F.; Guo, F. Impacts of essential amino acids on energy balance. *Mol Metab.* **2022**, *57*, 101393. DOI: 10.1016/j.molmet.2021.101393

54. Wiltafsky, M.K.; Schmidtlein, B.; Roth, F.X. Estimates of the optimum dietary ratio of standardized ileal digestible valine to lysine for eight to twenty-five kilograms of body weight pigs. *J. Anim. Sci.* **2009**, *87*, 2544–2553. DOI: 10.2527/jas.2008-1221

55. Salmon, W.D. The significance of amino acid imbalance in nutrition. *Am J Clin Nutr.* **1958**, *6*, 487–494. DOI: 10.1093/ajcn/6.5.487

56. 56. Fan, P.; Liu, P.; Song, P.; Chen, X.; Ma, X. Moderate dietary protein restriction alters the composition of gut microbiota and improves ileal barrier function in adult pig model. *Sci. Rep.* **2017**, *7*, 43412. DOI: 10.1038/srep43412

57. Pajarillo, E.A.B.; Chae, J.P.; Balolong, M.P.; Kim, H.B.; Kang, D.K. Assessment of fecal bacterial diversity among healthy piglets during the weaning transition. *J. Gen. Appl. Microbiol.* **2014**, *60*, 140–146. DOI: 10.2323/jgam.60.140

58. Kim, H.B.; Borewicz, K.; White, B.A.; Singer, R.S.; Sreevatsan, S.; Tu, Z.J.; Isaacson, R.E. Longitudinal investigation of the age-related bacterial diversity in the feces of commercial pigs. *Vet. Microbiol.* **2011**, *153*, 124–133. DOI: 10.1016/j.vetmic.2011.05.021

59. McCormack, U.M.; Curião, T.; Buzoianu, S.G.; Prieto, M.L.; Ryan, T.; Varley, P.; Crispie, F.; Magowan, E.; Metzler-Zebeli, B.U.; Berry, D.; O'Sullivan, O. Exploring a possible link between the intestinal microbiota and feed efficiency in pigs. *Appl. Environ. Microbiol.* **2017**, *83*, e00380–17. DOI: 10.1128/AEM.00380-17

60. Flint, H.J.; Bayer, E.A. Plant cell wall breakdown by anaerobic microorganisms from the mammalian digestive tract. *Ann. N.Y. Acad. Sci.* **2008**, *1225*, 280–288. DOI: 10.1196/annals.1419.022

61. Guevarra, R.B.; Hong, S.H.; Cho, J.H.; Kim, B.R.; Shin, J.; Lee, J.H.; Kang, B.N.; Kim, Y.H.; Wattanaphansak, S.; Isaacson, R.E.; Song, M. The dynamics of the piglet gut microbiome during the weaning transition in association with health and nutrition. *J. Anim. Sci. Biotechnol.* **2018**, *9*, 1–9. DOI: 10.1186/s40104-018-0269-6

62. Frese, S.A.; Parker, K.; Calvert, C.C.; Mills, D.A. Diet shapes the gut microbiome of pigs during nursing and weaning. *Microbiome* **2015**, *3*, 1–10. DOI: 10.1186/s40168-015-0091-8

63. Koo, B.; Kim, J.W.; Nyachoti, C.M. Nutrient and energy digestibility, and microbial metabolites in weaned pigs fed diets containing Lactobacillus–fermented wheat. *Anim. Feed Sci. Technol.* **2018**, *241*, 27–37. DOI: 10.1016/j.anifeedsci.2018.04.007

64. Marounek, M.I.L.A.N.; Fliegrova, K.A.T.E.R.I.N.A.; Bartos, S.T.A.N.I.S.L.A.V. Metabolism and some characteristics of ruminal strains of Megasphaera elsdenii. *Appl Environ Microbiol.* **1989**, *55*, 1570– 1573. DOI: 10.1128/aem.55.6.1570-1573.1989

65. Quan, J.; Cai, G.; Yang, M.; Zeng, Z.; Ding, R.; Wang, X.; Zhuang, Z.; Zhou, S.; Li, S.; Yang, H.; Li, Z. Exploring the fecal microbial composition and metagenomic functional capacities associated with feed efficiency in commercial DLY pigs. *Front. Microbiol.* **2019**, *10*, 52. DOI: 10.3389/fmicb.2019.00052

66. Yang, H.; Yang, M.; Fang, S.; Huang, X.; He, M.; Ke, S.; Gao, J.; Wu, J.; Zhou, Y.; Fu, H.; Chen, C. Evaluating the profound effect of gut microbiome on host appetite in pigs. *BMC Microbiol.* **2018**, *18*, 1–10. DOI: 10.1186/s12866-018-1364-8

67. Rattigan, R.; Sweeney, T.; Maher, S.; Ryan, M.T.; Thornton, K.; O'Doherty, J.V. Effects of reducing dietary crude protein concentration and supplementation with either laminarin or zinc oxide on the growth performance and intestinal health of newly weaned pigs. *Anim. Feed Sci. Technol.* **2020**, *270*, 114693. DOI: 10.1016/j.anifeedsci.2020.114693

68. Christensen, H.; Bossé, J.; Angen, Ø.; Nørskov-Lauritsen, N.; Bisgaard, M. Immunological and molecular techniques used for determination of serotypes in Pasteurellaceae. In *Book Immunological Methods in Microbiology*, 1st ed.; Pavia, C.S., Gurtler, V., Eds.; Academic Press: London, United Kingdom, 2020; 47, pp. 117–149. DOI: 10.1016/bs.mim.2020.01.002

69. Stetsko, T.I. Antibiotic resistance of bacteria of the family pasteurellaceae, pathogens of respiratory infections of cattle and pigs. *Sci. Tech. Bull. State Sci. Res. Con. Ins. Vet. Med. Pro. Fodder Add. Ins. Anim. Bio.* **2021**, *22*, 197–208. DOI: 10.36359/scivp.2021-22-1.24

70. Dou, S.; Gadonna-Widehem, P.; Rome, V.; Hamoudi, D.; Rhazi, L.; Lakhal, L.; Larcher, T.; Bahi-Jaber, N.; Pinon-Quintana, A.; Guyonvarch, A.; Huërou-Luron, I.L. Characterisation of early-life

fecal microbiota in susceptible and healthy pigs to post-weaning diarrhoea. *PLoS One*. **2017**, *12*, e0169851. DOI: 10.1371/journal.pone.0169851

71. Allen-Vercoe, E.; Jobin, C. Fusobacterium and Enterobacteriaceae: Important players for CRC? *Immunol. Lett.* **2014**, *162*, 54–61. DOI: 10.1016/j.imlet.2014.05.014

72. Guevarra, R.B.; Lee, J.H.; Lee, S.H.; Seok, M.J.; Kim, D.W.; Kang, B.N.; Johnson, T.J.; Isaacson, R.E.; Kim, H.B. Piglet gut microbial shifts early in life: causes and effects. *J. Anim. Sci. Biotechnol.* **2019**, *10*, 1–10. DOI: 10.1186/s40104-018-0308-3

73. Gresse, R.; Chaucheyras-Durand, F.; Fleury, M.A.; Van de Wiele, T.; Forano, E.; Blanquet-Diot, S. Gut microbiota dysbiosis in postweaning piglets: understanding the keys to health. *Trends Microbiol.* **2017**, *25*, 851–873. DOI: 10.1016/j.tim.2017.05.004

74. Rhouma, M.; Fairbrother, J.M.; Beaudry, F.; Letellier, A. Post weaning diarrhea in pigs: risk factors and non-colistin-based control strategies. *Acta Vet. Scand.* **2017**, *59*, 1–19. DOI: 10.1186/s13028-017-0299-7

75. Binda, C.; Lopetuso, L.R.; Rizzatti, G.; Gibiino, G.; Cennamo, V.; Gasbarrini, A. Actinobacteria: a relevant minority for the maintenance of gut homeostasis. *Dig. Liver Dis.* **2018**, *50*, 421–428. DOI: 10.1016/j.dld.2018.02.012

76. Bibbò, S.; Abbondio, M.; Sau, R.; Tanca, A.; Pira, G.; Errigo, A.; Manetti, R.; Pes, G.M.; Dore, M.P.; Uzzau, S. Fecal microbiota signatures in celiac disease patients with poly-autoimmunity. *Front. cell. infect.* **2020**, *10*, 349. DOI: 10.3389/fcimb.2020.00349

77. Mei, L.; Zhou, J.; Su, Y.; Mao, K.; Wu, J.; Zhu, C.; He, L.; Cui, Y. Gut microbiota composition and functional prediction in diarrhea-predominant irritable bowel syndrome. *BMC Gastroenterol.* **2021**, *21*, 1–12. DOI: 10.1186/s12876-021-01693-w

78. Tan, G.Y.; Liu, T. Rational synthetic pathway refactoring of natural products biosynthesis in actinobacteria. *Metab. Eng.* **2017**, *39*, 228–236. DOI: 10.1016/j.ymben.2016.12.006

79. Zeyner, A.; Boldt, E. Effects of a probiotic Enterococcus faecium strain supplemented from birth to weaning on diarrhoea patterns and performance of piglets. *J. Anim. Physiol. Anim. Nutr.* **2006**, *90*, 25–31. DOI: 10.1111/j.1439-0396.2005.00615.x

80. Bednorz, C.; Guenther, S.; Oelgeschläger, K.; Kinnemann, B.; Pieper, R.; Hartmann, S.; Tedin, K.; Semmler, T.; Neumann, K.; Schierack, P.; Bethe, A. Feeding the probiotic Enterococcus faecium strain NCIMB 10415 to piglets specifically reduces the number of Escherichia coli pathotypes that adhere to the gut mucosa. *Appl. Environ. Microbiol.* **2013**, *79*, 7896–7904. DOI: 10.1128/AEM.03138-13

81. Wang, Y.B.; Du, W.; Fu, A.K.; Zhang, X.P.; Huang, Y.; Lee, K.H.; Yu, K.; Li, W.F.; Li, Y.L. Intestinal microbiota and oral administration of Enterococcus faecium associated with the growth performance of new-born piglets. *Benef. Microbes.* **2016**, *7*, 529–538. DOI: 10.3920/BM2015.0099

82. Strompfova, V.; Marciňáková, M.; Simonová, M.; Gancarčíková, S.; Jonecová, Z.; Sciranková, Ľ.; Koščová, J.; Buleca, V.; Čobanová, K.; Lauková, A. Enterococcus faecium EK13—an enterocin A-producing strain with probiotic character and its effect in piglets. *Anaerobe* **2006**, *12*, 242–248. DOI: 10.1016/j.anaerobe.2006.09.003

83. Seddon, B.; McHugh, R.C.; Schmitt, A. Brevibacillus brevis-a novel candidate biocontrol agent with broad-spectrum antifungal activity. In The BCPC Conference: Pests and diseases, Volume 2. Proceedings of an international conference held at the Brighton Hilton Metropole Hotel, Brighton, UK, 13-16 November 2000, pp. 563–570. British Crop Protection Council, 2000.

84. Che, J.; Ye, S.; Liu, B.; Deng, Y.; Chen, Q.; Ge, C.; Liu, G.; Wang, J. Effects of Brevibacillus brevis FJAT-1501-BPA on growth performance, faecal microflora, faecal enzyme activities and

blood parameters of weaned piglets. *Anton. Leeuw. Int. J. Gen.* **2016**, *109*, 1545–1553. DOI: 10.1007/s10482-016-0756-8

85. Zhu, L.; Liao, R.; Tu, W.; Lu, Y.; Cai X. Pyrodextrin enhances intestinal function through changing the intestinal microbiota composition and metabolism in early weaned piglets. *Appl Microbiol Biotechnol*. **2020**, *104*, 4141–4154. DOI: 10.1007/s00253-020-10419-z.

CHAPTER V

DIETARY ISOLEUCINE AND VALINE: EFFECTS ON LIPID METABOLISM AND UREAGENESIS IN PIGS FED WITH PROTEIN RESTRICTED DIETS

This chapter is based on: Parniyan Goodarzi, Mohammad Habibi[1], Matthew William Gorton, Katherine Walsh, Firoozeh Tarkesh, Mallory Fuhrig and Adel Pezeshki. Dietary isoleucine and valine: effects on lipid metabolism and ureagenesis in pigs fed with protein restricted diets.

5.1. Abstract

The objective of this study was to investigate the effect of dietary valine (Val) and isoleucine (Ile) on gene and protein expression of key rate limiting enzymes of urea cycle and lipid metabolism in pigs fed with very low protein (VLP) diets. Forty, three-week-old, weaned pigs were assigned to 5 treatments: positive control (PC): normal protein diet; negative control (NC): VLP diet containing first four limiting amino acids; HV: NC supplemented with Val above NRC; HI: NC supplemented with Ile above NRC; HVI: NC supplemented with both Val and Ile above NRC. HVI partially improved the body weight and completely recovered the feed intake of pigs fed with VLP diets. HVI increased the thermal radiation and improved the glucose clearance following a meal test. HVI had a lower blood triglyceride than PC and blood urea nitrogen than NC. Pigs fed with NC and HV promoted lipogenesis with increasing the mRNA abundance of hepatic FAS and SREBP1 and adipose LPL but reducing transcript of hepatic HSL and PGC1α.

HVI reduced the increased rate of lipogenesis induced by VLP groups through normalizing the transcript of hepatic FAS, SREBP1, HSL and PGC1α and LPL in adipose tissue. All low protein groups (*i.e.*, NC, HV, HI and HVI) reduced the ureagenesis through decreasing the protein expression of rate-limiting enzymes of ureagenesis, CPS1, OTC and ASL, in liver. Overall, a combination of dietary Val and Ile improved the growth, feed intake, and glucose clearance and decreased the rate of lipogenesis induced by VLP diets.

5.2. Introduction

Excess nitrogen (N) excreted from modern swine production has a negative impact on the environment through contributing to acidification and eutrophication of sensitive ecosystems and odor emissions [1]. Reducing dietary crude protein (CP) by more than 4%-unit has been shown to increase the N utilization [2-4], but growth performance is depressed even when first four limiting amino acids (*i.e.*, Lys, Met, Thr and Trp) are supplemented in the diet of early weaned, growing and finishing pigs [5-8].

We have previously demonstrated that supplementing very low protein (VLP) diets with both limiting amino acids (AA) and branched-chain amino acids (BCAA, *i.e.*, Leu, Ile and Val) or mixture of Ile and Val not only decreases the negative impact of these diets on growth but also reduce the blood urea N (BUN) [9-11]. Others have shown that dietary supplementation of Val [12-14] and Ile [15] reduce BUN in pigs. Given a positive correlation between lower BUN and decreased N excretion in pigs [16, 17], it appears that BCAA promote N retention. There is evidence that dietary BCAA may potentially increase the efficiency of AA and N utilization in pigs [18, 19], and humans [20-22]. Little is understood on the mechanisms by which BCAA influence the N balance. Nitrogen balance is the results of dynamic protein digestion, absorption, and metabolism. Dietary BCAA improve the N utilization possibly through increasing the activity and/or secretion rate of proteolytic enzymes [23-26] and upregulation of intestinal AA transporters

[27-29] and providing N for de novo synthesis of AA [30-32]. The effect of Val and Ile alone or in combination on expression of urea cycle enzymes is yet to be understood.

Our previous data provide evidence on the role of dietary BCAA on lipid metabolism in pigs fed with VLP diets [9, 33]. The literature on the regulatory role of BCAA on lipid metabolism is equivocal. Evidences show that dietary supplementation of BCAA stimulates lipolysis and reduces fat deposition, white adipose tissue mass and TG concentration in muscle and liver in mice [34-37]. Despite this, others have reported a lipogenic role for BCAA increasing the serum TG and fat accumulation in white adipose tissue in mice [38, 39], but their restriction or deprivation promote fat loss and reduce the organs TG content in rats, mice and broilers [40-44]. While pigs have many similarities to humans in terms of metabolism, dietary habits, nutritional requirements, and nutrients interactions [45, 46] and have been previously used as a model for studying the AA metabolism [47, 48] and metabolic complications [49, 50], little is understood on the role of BCAA on lipid metabolism in pigs. To our knowledge no study has examined the effect of a combination of Val and Ile on lipid metabolism in pigs offered with VLP diets. The objective of this study was to investigate the effect of dietary Val and Ile on gene and protein expression of key rate limiting enzymes of urea cycle and lipid metabolism in pigs fed with VLP diets.

5.3. Materials and methods

5.3.1. Animals and housing

All the experimental procedures used in this study were reviewed and approved by Oklahoma State University's Institutional Animal Care and Use Committee approved (IACUC-20-54). A total of forty, three weeks old, weaned barrows (Duroc sire line and Large White × Landrace dam) with the average body weight (BW) of 6.10 ± 0.62 kg were used (Seaboard, Hennessey, OK). Upon arrival, animals were group housed in an environmentally controlled animal room equipped with

feeders and waters as we previously described [11]. Feed was provided *ad libitum* and all pigs had free access to water during the study.

5.3.2. Diets and experimental design

After one week of adaption, pigs were weight-matched (average BW of 6.98 ± 0.80 kg) and randomly assigned to 5 dietary treatments (n=8/group) for 5 weeks including: 1) positive control (PC): standard protein diet; 2) negative control (NC): very low protein diet containing first four limiting amino acids (*i.e.*, Lys, Met, Thr and Trp) at NRC (2012) [51] levels; 3) HV: NC containing standard ileal digestibility (SID) Val: Lys ratio of 0.75; 4) HI: NC containing SID Ile: Lys ratio of 0.60; 5) HVI: NC containing SID Val: Lys ratio of 0.75 and SID Ile: Lys ratio of 0.60. The used ratios for SID Val:Lys and Ile:Lys were based on previous literature reporting an improved performance of pigs fed with low protein diets with above values for added Val and Ile [16, 52] . Using National Swine Nutrition Guide (Version 2.1 Metric, ©2012 U.S. Pork Center of Excellence) and NRC recommendations (NRC, 2012) [51] for animals' nutritional requirements at different ranges of BW, three nursery phase diets were formulated. The nursery phase 1 (N1), phase 2 (N2), and phase 3 (N3) were fed on days 1-7, 8-21, and 22-42, respectively. All diets were formulated to be isocaloric by using variable amounts of corn and soybean meal. Further, L-Alanine was used to keep the NC, HV, HI, and HVI diets isonitrogenous. The amounts of other ingredients used were kept as consistent as possible. The ingredients and chemical composition of all diets are given in Table 5.1.

Table 5.1. Ingredients and chemical composition of experimental diets (as-fed basis)

	Diets [1]									
	N1	N2					N3				
Ingredients [2], %		**PC**	**NC**	**HV**	**HI**	**HVI**	**PC**	**NC**	**HV**	**HI**	**HVI**
Corn, yellow dent	37.41	55.60	75.54	75.41	75.40	75.27	69.16	87.14	87.02	87.01	86.89
Soybean meal, 47.5% CP	18.00	21.67	2.42	2.42	2.42	2.42	18.60	1.10	1.10	1.10	1.10
Fish meal, menhaden	6.00	4.29	4.29	4.29	4.29	4.29	4.29	4.29	4.29	4.29	4.29
Whey, dried	24.10	3.00	3.00	3.00	3.00	3.00	—	—	—	—	—
Corn starch	—	9.43	6.92	6.92	6.92	6.92	3.25	1.16	1.16	1.16	1.16
Lactose	6.80	—	—	—	—	—	—	—	—	—	—
Plasma spray-dried	5.29	3.10	3.10	3.10	3.10	3.10	2.10	2.00	2.00	2.00	2.00
Corn oil	0.49	—	—	—	—	—	—	—	—	—	—
Dicalcium phosphate 18.5%	0.86	1.25	1.63	1.63	1.63	1.63	1.05	1.41	1.41	1.41	1.41
Limestone	0.38	0.48	0.36	0.36	0.36	0.36	0.40	0.31	0.31	0.31	0.31
Salt	0.16	0.52	0.52	0.52	0.52	0.52	0.47	0.47	0.47	0.47	0.47
Vitamin premix	0.04	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Trace mineral premix	—	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01
Zinc oxide, 72% Zn	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
L-Lysine, HCl	0.27	0.36	0.85	0.85	0.85	0.85	0.38	0.84	0.84	0.84	0.84
DL-methionine	0.13	0.11	0.19	0.19	0.19	0.19	0.09	0.16	0.16	0.16	0.16
L-threonine	0.05	0.10	0.36	0.36	0.36	0.36	0.12	0.36	0.36	0.36	0.36
L-tryptophan	0.01	0.01	0.11	0.11	0.11	0.11	0.02	0.11	0.11	0.11	0.11
L-isoleucine	—	—	—	—	0.42	0.42	—	—	—	0.39	0.39
L-valine	—	—	—	0.48	—	0.48	—	—	0.44	—	0.44
L-alanine	—	—	0.63	0.28	0.35	—	—	0.58	0.26	0.32	—
Calculated Chemical Composition [3]											
Dry matter, %	90.32	90.00	89.63	89.65	89.65	89.67	88.81	88.70	88.74	88.74	88.76
ME, Mcal/kg	3.40	3.40	3.40	3.40	3.40	3.40	3.35	3.35	3.35	3.35	3.35
Crude protein, %	22.00	20.08	14.00	14.00	14.00	14.00	18.60	13.00	13.00	13.00	13.00
Crude fiber, %	1.67	2.25	1.86	1.86	1.86	1.86	2.42	2.07	2.06	2.06	2.06
Crude fat, %	3.36	3.07	3.40	3.39	3.39	3.39	3.44	3.72	3.72	3.72	3.71
Calcium, %	0.85	0.80	0.80	0.80	0.80	0.80	0.70	0.70	0.70	0.70	0.70

Total phosphorus, %	0.70	0.65	0.65	0.65	0.65	0.65	0.60	0.60	0.60	0.60	0.60
Available phosphorus, %	0.61	0.47	0.52	0.52	0.52	0.52	0.40	0.45	0.45	0.45	0.45
SID Lysine, %	1.50	1.35	1.35	1.35	1.35	1.35	1.23	1.23	1.23	1.23	1.23
SID Threonine, %	0.88	0.79	0.79	0.79	0.79	0.79	0.73	0.73	0.73	0.73	0.73
SID Methionine, %	0.43	0.39	0.39	0.39	0.39	0.39	0.36	0.36	0.36	0.36	0.36
SID Tryptophan, %	0.25	0.22	0.22	0.22	0.22	0.22	0.20	0.20	0.20	0.20	0.20
SID Isoleucine, %	0.79	0.71	0.39	0.39	0.81	0.81	0.64	0.35	0.35	0.74	0.74
SID Valine, %	0.96	0.86	0.53	1.01	0.53	1.01	0.78	0.48	0.92	0.48	0.92
SID Leucine, %	1.65	1.52	1.08	1.08	1.08	1.08	1.44	1.03	1.03	1.03	1.03
SID Histidine, %	0.50	0.47	0.30	0.30	0.30	0.30	0.44	0.28	0.28	0.28	0.28
SID Arginine, %	1.14	1.14	0.59	0.59	0.59	0.59	1.04	0.54	0.54	0.54	0.53
SID Phenylalanine, %	0.90	0.85	0.50	0.50	0.50	0.50	0.78	0.46	0.46	0.46	0.46
SID Valine: SID Lysine	0.64	0.64	0.39	0.75	0.39	0.75	0.63	0.39	0.75	0.39	0.75
SID Isoleucine: SID Lysine	0.52	0.53	0.29	0.29	0.60	0.60	0.52	0.28	0.28	0.60	0.60

[1]Diets were formulated using National Swine Nutrition Guide (NSNG; Version 2.1 Metric, ©2012 U.S. Pork Center of Excellence). PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (*i.e*., lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; HV: NC containing valine (Val) above NRC level; HI: NC containing isoleucine (Ile) above NRC level; HVI: NC containing both Val and Ile above NRC level. N1 (nursery phase 1): this diet was provided from day 1 to 7 of the study; N2 (nursery phase 2): these diets were fed from day 8 to 21 of the study and N3 (nursery phase 3): these diets were offered from day 22 to 42 of the study.

[2]Corn, soybean meal, fish meal, whey, corn starch, lactose, plasma spray-dried, corn oil, dicalcium phosphate, limestone, salt, zinc oxide, vitamin premix, trace mineral premix, DL-methionine (99%) and L-lysine HCl (79-99%) were purchased by Nutra Blend, LLC (Neosho, MO). L-threonine (98.5%) and L-tryptophan (98%) were purchased from Ajinomoto (Overland Park, KS). L-isoleucine (98.5%), L-alanine and L-valine (96.5%) were provided from Ajinomoto Health & Nutrition North America, Inc. (Raleigh, NC). Vitamin premix contained: vitamin A, 1,653,750 IU/kg; vitamin D3, 661,500 IU/kg; vitamin E, 17,640 IU/kg; vitamin

K (menadione), 1,323 mg/kg; vitamin B12, 13.23 mg/kg; niacin, 19,845 mg/kg; D-pantothenic acid, 11,025 mg/kg; riboflavin, 3,307.5 mg/kg; phytase, 300,056.4 FYT/kg. Trace mineral premix contained: copper, 11,000 ppm; iodine, 198 ppm; iron, 73,000 ppm; manganese, 22,000 ppm; selenium, 198 ppm; zinc, 73,000 ppm.

[3]ME: metabolize energy; SID: standard ileal digestibility

5.3.3. Body weight, feed intake, and water intake

Individual feed intake (FI) and water intake were monitored daily. Further, FI was measured at 3, 6, 9, 12, and 24 hours after feeding at 0800 biweekly. Body weight and growth parameters including body length, wither height, and heart girth of all pigs were recorded weekly. Average daily gain (ADG), average daily feed intake (ADFI), average daily protein intake (ADPI), average daily water intake (ADWI), gain-to-feed ratio (G:F), gain-to-protein ratio (G:P), water-to-feed ratio (W:F), body weight gain (BWG), mean feed intake (MFI), cumulative feed intake (CFI), and cumulative protein intake (CPI), were calculated by using BW, FI and WI data.

5.3.4. Thermal imaging

Weekly thermal images were acquired about 1 m above each pig (emissivity coefficient of 0.95) using a FLIR C2 compact thermal camera with a focal length of 1.54 mm and a thermal accuracy of ±2 ºC (FLIR Systems, Boston, MA, USA). Representative thermal images for each of dietary groups are shown in Fig. 5A.1.

5.3.5. Feed, blood, and tissue samples collection

The feed samples (about 50 g) were taken from each feed bag and pooled for each diet during the diet preparation. The samples were then stored at -20 °C until feed composition analysis. At week 6, pigs were allowed to consume their respective diets for one hour following overnight fast (~ 8 hours), and then blood samples were drawn from the jugular vein at baseline and then at 60, and 120 minutes after the meal test. The blood samples were collected in 10.0 mL serum tubes and 3.0 mL plasma tubes containing lithium heparin (BD Vacutainer®, Franklin Lakes, NJ, USA). Blood samples were centrifuged at 3,000 × g for 15 minutes at 4 ºC, serum or plasma was separated and stored at −80 °C. All pigs were euthanized using the CO2 asphyxiation method 120 minutes after the meal test, the liver and kidney samples were immediately extracted, snap-frozen in liquid nitrogen, and stored at −80 °C.

5.3.6. Thermal radiation analysis

The mean dorsal body surface temperature was obtained by drawing a rectangle in the entire back of pigs using FLIR camera software (FLIR Research Studio software, FLIR Systems, Boston, MA, USA) as we previously described [53]. The following equation was then used to calculate the thermal radiation (W/m^2): $\sigma\varepsilon\ (T_s^4 - T_\alpha^4)$ where σ is Stefan Boltzmann constant (5.67×10^{-8} W / m^2K^4), ε is thermodynamic emissivity (0.95), T_s is mean body surface temperature (kelvin) and T_α is ambient temperature (kelvin).

5.3.7. Diets composition analysis

The chemical composition (i.e., dry matter, CP, crude fiber, calcium, and phosphorus) and amino acids concentrations of experimental diets were analyzed by ServiTech laboratories (Dodge City, KS) [7, 33, 54] and Agricultural Experiment Station Chemical Laboratories (University of Missouri-Columbia, MO) [53], respectively. The results of diets composition and amino acids analysis are given in Table 5.2.

Table 5.2. Analyzed chemical composition of experimental diets (as-fed basis)

Chemical composition	Diets[1]									
	N1	N2					N3				
		PC	NC	HV	HI	HVI	PC	NC	HV	HI	HVI
Dry matter, %	91.30	89.30	89.10	89.50	89.10	89.30	88.80	88.30	88.70	88.50	88.70
Crude protein, %	20.40	19.50	13.50	13.60	14.00	13.80	19.30	12.30	12.70	13.80	12.60
Crude fiber, %	1.40	2.10	1.50	1.50	1.80	1.70	2.00	1.80	2.10	2.30	2.30
Calcium, %	0.95	0.81	0.77	0.77	0.80	0.75	0.83	0.71	0.85	0.80	0.80
Phosphorus, %	0.87	0.72	0.68	0.69	0.77	0.68	0.72	0.61	0.67	0.68	0.73
Taurine[2], %	0.19	0.19	0.19	0.19	0.19	0.21	0.19	0.20	0.21	0.19	0.20
Hydroxyproline, %	0.18	0.12	0.10	0.10	0.11	0.00	0.15	0.11	0.00	0.12	0.12
Aspartic acid, %	2.16	1.96	1.05	1.03	1.05	1.05	1.76	0.91	0.90	0.96	0.84
Threonine, %	1.09	0.90	0.84	0.92	0.95	0.91	0.85	0.77	0.87	0.76	0.70
Serine, %	0.96	0.88	0.54	0.54	0.55	0.61	0.80	0.48	0.51	0.51	0.47
Glutamic acid, %	3.45	3.37	1.96	1.93	1.97	2.02	3.04	1.79	1.83	1.92	1.73
Proline, %	1.28	1.22	0.86	0.83	0.86	0.82	1.14	0.80	0.81	0.84	0.78
Lanthionine[2], %	0.07	0.04	0.03	0.04	0.03	0.00	0.05	0.03	0.00	0.04	0.03
Glycine, %	0.96	0.91	0.59	0.59	0.60	0.61	0.85	0.55	0.54	0.56	0.52
Alanine, %	1.12	1.03	1.37	1.04	1.08	0.77	0.99	1.41	0.99	1.10	0.69
Cysteine, %	0.46	0.39	0.26	0.25	0.26	0.26	0.34	0.23	0.22	0.24	0.20
Valine, %	1.16	1.01	0.64	1.11	0.65	1.14	0.90	0.56	0.97	0.59	0.91
Methionine, %	0.52	0.48	0.38	0.43	0.38	0.40	0.42	0.37	0.39	0.33	0.31
Isoleucine, %	0.92	0.83	0.47	0.45	0.86	0.93	0.74	0.41	0.41	0.80	0.74
Leucine, %	1.82	1.67	1.15	1.13	1.16	1.17	1.56	1.06	1.07	1.12	1.04
Tyrosine, %	0.72	0.65	0.35	0.36	0.37	0.40	0.59	0.33	0.33	0.32	0.33
Phenylalanine, %	0.97	0.95	0.57	0.56	0.57	0.57	0.87	0.51	0.50	0.53	0.49
Hydroxylysine, %	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01
Ornithine[2], %	0.02	0.02	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01
Lysine, %	1.71	1.53	1.38	1.60	1.47	1.47	1.40	1.33	1.31	1.30	1.28
Histidine, %	0.54	0.51	0.32	0.32	0.32	0.33	0.47	0.29	0.29	0.31	0.28
Arginine, %	1.19	1.20	0.64	0.63	0.64	0.67	1.09	0.57	0.57	0.60	0.54
Tryptophan, %	0.29	0.27	0.22	0.23	0.24	0.21	0.23	0.19	0.20	0.20	0.19

Valine: Lysine	0.68	0.66	0.46	0.69	0.44	0.78	0.64	0.42	0.74	0.45	0.71
Isoleucine: Lysine	0.54	0.54	0.34	0.28	0.59	0.63	0.53	0.31	0.31	0.62	0.58

[1]PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (*i.e*., lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; HV: NC containing valine (Val) above NRC level; HI: NC containing isoleucine (Ile) above NRC level; HVI: NC containing both Val and Ile above NRC level. N1 (nursery phase 1): this diet was provided from day 1 to 7 of the study; N2 (nursery phase 2): these diets were fed from day 8 to 21 of the study and N3 (nursery phase 3): these diets were offered from day 22 to 42 of the study.

[2]Non-proteinogenic amino acids.

5.3.8. Plasma metabolites and urea analysis

The plasma glucose, triglyceride, and cholesterol concentration were determined by a chemistry analyzer (R404200-3, Alfa Wassermann's Vet Axcel, West Caldwell, NJ) following calibration with a calibrator (BL-442600, Multi-Analyte calibrator for Synchron CX/ LX) and using reagents (Carolina Liquid Chemistries Crop, Brea, CA) for glucose (BL-208), cholesterol (BL-211), and triglyceride (BL-213). Absorbance was recorded at 340 nm for glucose and at 505 nm for triglyceride and cholesterol. QuantiChrom^{TM} Urea Assay Kit (DIUR-100, BioAssay Sytems, Hayward, CA) was used to detect plasma urea concentration according to the manufacturer's instructions. The optical density was measured with an Epoch microplate spectrophotometer (BioTek® Instruments, Inc. Highland Park, VT) at a wavelength of 520 nm. The intra-assay coefficient of variation was 9.73%.

5.3.9. Reverse transcription, and quantitative PCR (RT-qPCR)

Following our published procedures [10, 33, 55, 56], RNA was isolated and RT-qPCR was performed for carnitine palmitoyltransferase 1 α (CPT1 α), lipoprotein lipase (LPL), cluster of differentiation 36 molecule (CD36), fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), hormone-sensitive lipase (HSL), hydroxyacyl-CoA dehydrogenase (HADH), sterol regulatory element binding transcription factor 1 (SREBP-1), peroxisome proliferator activated receptor alpha (PPARα), and PPARG coactivator 1 alpha (PGC1α) in liver and subcutaneous adipose tissue. The primer sequences were obtained from other studies [57-63]. Details on primers used are listed in Table 5A.1. The relative abundances of target genes transcript were calculated using Ct values for target and housekeeping (Table 5A.1) genes using $2^{-\Delta\Delta CT}$ method.

5.3.10. Immunoblot analysis

Western blots were performed in the liver and kidney for carbamoyl phosphate synthetase I (CPS I), ornithine transcarbamylase (OTC), argininosuccinate synthase 1 (ASS 1), arginase 1 (ARG 1),

and argininosuccinate lyase (ASL) as we previously described [10, 33, 64]. β-actin and GAPDH were employed as loading controls to establish the relative amount of target protein abundance. The details on antibodies used are given in Table 5A.2.

5.3.11. Statistical analysis

Overall growth, cumulative hourly FI, thermal radiation, and all other data obtained from laboratory analyses, including plasma metabolites, RT-qPCR, and western blot data were analyzed by GLM procedure with Tukey post-hoc test following an outlier test which was based on the Interquartile Rule (IBM SPSS Statistics Version 23, Armonk, NY, USA). The hourly, daily, and weekly recorded data, including FI, water intake, BW, BWG, MFI, CFI, CPI, G:F, and G:P, were subjected to a mixed analysis with the diet, time, and interaction of diet by time as fixed effects and the animal as a random variable in the model. The lowest quantities of fit statistics for corrected Akaike Information Criterion and Bayesian Information Criterion were used to model covariance structure for repeated measurements for each variable. For plasma glucose after a meal test a paired Student's t-test followed by a Benjamini-Hochberg correction with 0.1 false discovery rate was used to determine the differences between means of five preplanned comparisons: PC 0 min vs. PC 120 min, NC 0 min vs. NC 120 min, HV 0 min vs. HV 120 min, HI 0 min vs. HI 120 min, HVI 0 min vs. HVI 120 min. Differences among treatments were considered significant at $P \leq 0.05$ and a trend at $0.05 < P \leq 0.10$.

5.4. Results

5.4.1. Growth measurements

No differences on initial BW of animals were seen among groups (Tables 5.3). Compared to PC, NC, HV, HI and HVI had a lower final BW (39, 37, 35 and 19%, respectively). HVI had a higher final BW than NC, HV, and HI by 34, 30, and 26%, respectively. Relative to PC, NC, HV, and HI reduced the ADG by 50, 50, and 47%, respectively (Table 5.3). The ADG of pigs fed with HVI

was lower (24%) than PC and that was 52, 52 and 42% higher than NC, HV and HI, respectively. The effect of diet on BWG was significant in all weeks (Table 5A.3). Relative to PC, NC reduced the BW by 16-39% throughout the study ($P < 0.05$; Fig. 5.1A). HV and HI had a lower BW than PC on days 14-35 ($P < 0.01$; Fig. 5.1A). HVI and PC had a similar BW on days 7 and 14 but HVI had a 14-19% lower BW than PC in the last three weeks of study. While HI and HV had a similar BW as NC, HVI had a 21-37% higher BW than NC on days 14-35. Relative to HV and HI, HVI had a higher BW on days 14-35 ($P < 0.01$; Fig. 5.1A). BW of HV was not changed when compared to HI in the entire study (Fig. 5.1A).

Compared to PC, NC, HV, and HI reduced the ADFI by 38, 26, and 33%, respectively, but HVI was not different compared to PC (Table 5.3). Pigs fed with HV and HI had similar ADFI as NC while HVI had 47% higher ADFI than NC. Further, HVI had 34% higher ADFI than HI (Table 5.3). Compared with PC, pigs fed with NC, HV, HI and HVI had a lower ADPI. While ADPI for HV and HI was not different compared to NC, pigs fed with HVI had 43% higher ADPI than NC. Further, HVI tended to have a higher ADPI than HI. The effect of diet on MFI, CFI and CPI was significant in all weeks (Table 5A.3). Compared to PC, pigs fed with NC reduced the FI on day 9 onward (33-46%) ($P < 0.05$; Fig. 5.1B). HV had a lower or tended to have a lower FI than PC on days 14, 16, 19, 23 and 35 and HI either had a lower or tended to have a lower FI than PC on days 7, 11, 16, 19, 21, 27, 29, 33, and 35 (Fig. 5.1B). Pigs fed with HVI had a similar FI compared to PC on most of experimental days (Fig. 5.1B). Relative to NC, FI of HV and HI was not different on day 7 onward (Fig. 5.1B). HVI had a higher and tended to have a higher FI than NC on days 11, 16, 19, 23 and 27 (Fig. 5.1B). FI of HVI was not different relative to HV in the entire experiment except for day 19 (Fig. 5.1B). Pigs fed with HI had a lower FI relative to HVI on days 7, 11, 19, and 27 ($P < 0.05$; Fig. 5.1B). The effect of dietary treatments on hourly FI on some representative experimental days is shown in Fig. 5A.2.

ADWI was lower in NC, HV, and HI compared to PC (45, 26, 33, and 24%, respectively); however, HVI had a similar ADWI as PC (Table 5A.3). Water intake was either lower or tended to be lower in pigs fed with NC compared to PC on days 7, 11, 14, 23, 25, 27, 30, and 33 ($P < 0.05$; Fig. 5.1C). HV had a lower water intake than PC on days 23, 25 and 27 ($P < 0.05$; Fig. 5.1C). Water intake was either lower or tended to be lower in pigs fed with HI than PC on days 7, 25, 27, 30, 33 and 35 ($P < 0.05$; Fig. 5.1C). HVI had a similar water intake compared to PC except day 27 when the water intake tended to be lower for HVI ($P < 0.05$; Fig. 5.1C).

Compared with PC, pigs fed with NC, HV, HI and HVI had 19, 26, 19 and 18% lower G:F, respectively ($P < 0.01$; Table 5.3). No differences among groups were found when G:F of NC was compared to HV, HI, and HVI ($P > 0.1$; Table 5.3). HVI tended to have a higher G:F than HV. Compared with PC, G:P ratio was higher in pigs fed with NC and HVI and tended to be higher in HI. Relative to NC, HI and HVI had similar G:P while HV had a lower G:P. HVI tended to have a higher G:P than HV (Table 5.3). There was no difference among treatments on W:F ratio (Table 5.3). Weekly G:F and G:P are shown in Table 5A.3.

Compared to PC, body length was lower in NC, HV, and HI; however, it was not different with HVI (Table 5.3). Relative to NC, HV and HI had a similar body length while it was higher in HVI by 11%. Relative to HV, and HI, the body length of HVI group was higher by 7 and 11%, respectively. HV and HI had similar body length. Heart girth was lower in NC, HV, HI and HVI than PC by 17, 17, 14, and 8%, respectively (Table 5.3). Relative to NC, no differences in heart girth were detected for HV and HI, but HVI had higher heart girth by 11%. Further, heart girth of HVI was 11% higher than HV. While relative to PC, wither height was lower in pigs fed with NC, HV, and HI (14, 16, and 14%, respectively), no differences in wither height were seen between HVI and PC (Table 5.3). Compared to NC, HV, HI, and HVI were not different, but HVI had a higher wither height than HV.

Table 5.3. Growth performance of nursery pigs fed with very low-protein diets containing isoleucine, valine or mix of both above NRC levels

Measurements[2]	Diets[1]					SEM[3]	*P*-value
	PC	NC	HV	HI	HVI		
Initial BW, kg	6.99	7.10	6.86	7.00	6.96	0.13	0.99
Final BW, kg	27.45^{a}	16.64^{c}	17.21^{c}	17.76^{c}	22.31^{b}	0.78	< 0.01
ADG, kg/day	0.58^{a}	0.29^{c}	0.29^{c}	0.31^{c}	0.44^{b}	0.02	< 0.01
ADFI, kg/day	0.86^{a}	0.53^{c}	0.64^{bc}	0.58^{c}	0.78^{ab}	0.03	< 0.01
ADPI, kg/day	0.17^{a}	0.07^{c}	0.08^{bc}	0.08^{bc}	0.10^{τ}	0.01	< 0.01
ADWI, L/day	2.36^{a}	1.31^{b}	1.74^{b}	1.58^{b}	1.80^{ab}	0.08	< 0.01
G:F, kg/kg	0.68^{a}	0.55^{b}	0.50^{b}	0.55^{b}	0.56^{ε}	0.01	< 0.01
G:P, kg/kg	3.54^{a}	4.37^{b}	3.83^{ca}	$3.98^{bc\phi}$	$4.31^{b\varepsilon}$	0.07	< 0.01
W:F , L/kg	2.77	2.40	2.72	2.73	2.40	0.07	0.26
Final body length, m	0.64^{a}	0.55^{b}	0.57^{b}	0.55^{b}	0.61^{a}	0.01	< 0.01
Final heart girth, m	0.66^{a}	0.55^{c}	0.55^{c}	0.57^{bc}	0.61^{b}	0.01	< 0.01
Final wither height, m	0.44^{a}	0.38^{bc}	0.37^{c}	0.38^{bc}	$0.41^{a\tau}$	0.00	< 0.01

[1]PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (*i.e.*, lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; HV: NC containing valine (Val) above NRC level; HI: NC containing isoleucine (Ile) above NRC level; HVI: NC containing both Val and Ile above NRC level. The values are means, n=8.

[2]BW: body weight; ADG: average daily gain; ADFI: average daily feed intake; ADPI: average daily protein intake; ADWI: average daily water intake; G:F: gain:feed ratio; G:P: gain:protein ratio; W:F: water:feed ratio.

[3]SEM: standard error of the mean.

[a b,c,d] Within each row, the values with different superscript letter(s) are different ($P \leq 0.05$). [τ] $P \leq 0.1$ HVI vs. HI, [ε] $P \leq 0.1$ HVI vs. HV, [ϕ] $P \leq 0.1$ HI vs. PC.

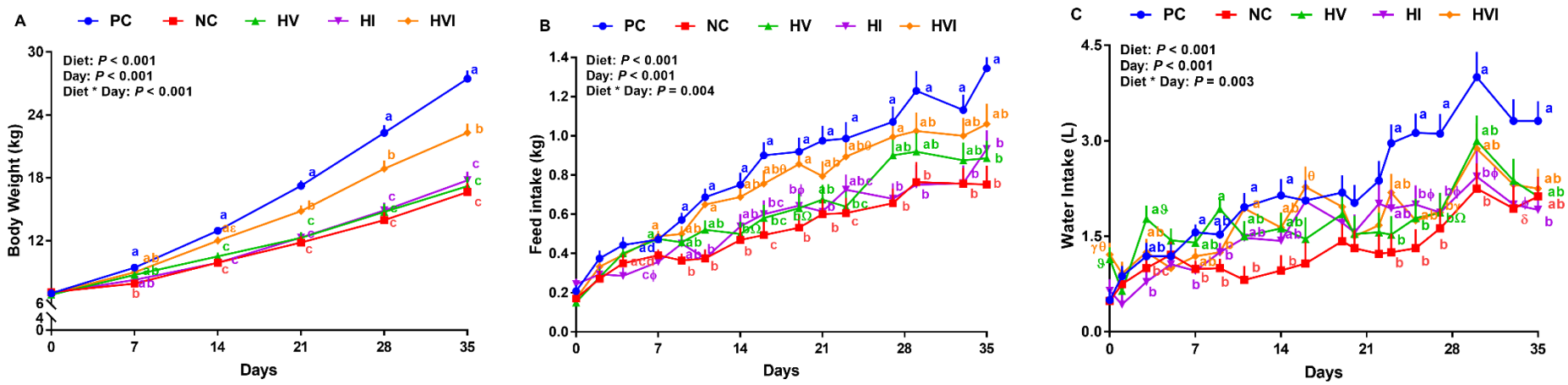


Figure 5.1. (A) Body weight, (B) feed intake, and (C) water intake of nursery pigs fed with very low protein diets containing isoleucine (Ile), valine (Val) or mix of both above NRC levels.

PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (*i.e.*, lysine, methionine, threonine and tryptophan) at NRC (2012) levels; HV: NC containing Val above NRC level; HI: NC containing Ile above NRC level; HVI: NC containing both Val and Ile above NRC level. The values are means ± standard error of the mean. n=8. ^{a,b,c,ab} the means with different superscript letter(s) at each time point are different ($P \leq 0.05$). $^{\delta}$ $P \leq 0.1$ PC vs. NC, $^{\Omega}$ $P \leq 0.1$ HV vs. PC, $^{\gamma}$ $P \leq 0.1$ HVI vs. PC, $^{\phi}$ $P \leq 0.1$ HI vs. PC, $^{\varepsilon}$ $P \leq 0.1$ HVI vs. HV, $^{\vartheta}$ $P \leq 0.1$ HV vs. NC, $^{\theta}$ $P \leq 0.1$ HVI vs. NC.

5.4.2. Thermal radiation

Overall, the effects of diet, day, and the interaction of diet by day on thermal radiation were significant ($P < 0.05$; Fig. 5.2A). No significant differences on thermal radiation of different groups were detected during the first two weeks. Relative to PC, NC reduced the thermal radiation by 12, 8, and 4%, on weeks 3 to 5, respectively (Fig. 5.2A). Thermal radiations of HV, HI and HVI were not different compared to PC throughout the study except for HI that had a higher thermal radiation on day 35. Thermal radiation of HI was higher than NC on weeks 3 and 5 by 9 and 16%, respectively ($P < 0.01$; Fig. 5.2A). Relative to NC, HVI had a higher thermal radiation by 10% on weeks 3 to 5. Relative to PC, thermal radiation of dietary treatments did not change (Fig. 5.2B). Relative to NC, the AUC thermal radiation of HVI was higher by 8% ($P < 0.01$; Fig. 5.2B). Furthermore, the AUC thermal radiation tended to increase in HV in comparison with NC. No differences in AUC thermal radiation were detected when HVI, HI and HVI groups were compared.

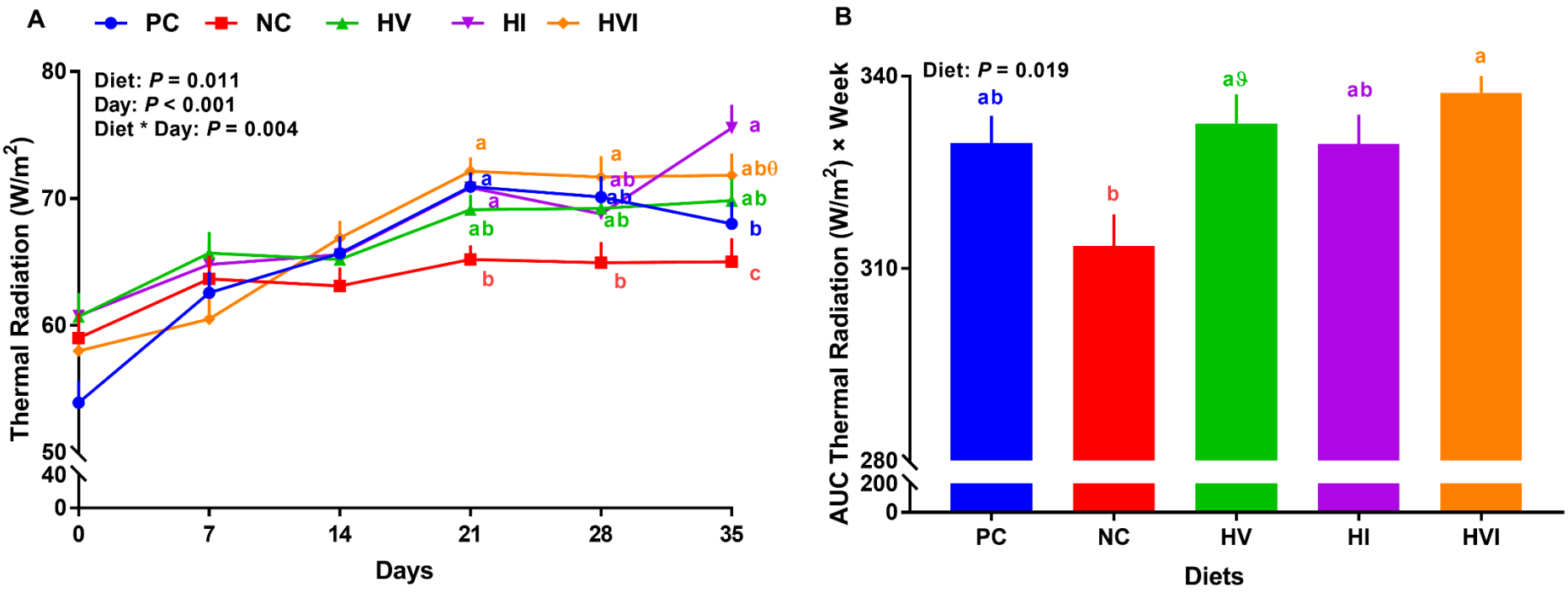


Figure 5.2. (**A**) Thermal radiation and (**B**) area under the curve (AUC) for thermal radiation in nursery pigs fed with very low protein diets containing isoleucine (Ile), valine (Val) or mix of both above NRC levels.

PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (*i.e.*, lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; HV: NC containing Val above NRC level; HI: NC containing Ile above NRC level; HVI: NC containing both Val and Ile above NRC level. The values are means ± standard error of the mean. n=8. ^{a,b,ab} Among groups, the means with different superscript letter(s) at each time point are different ($P \leq 0.05$). $^{\vartheta}P \leq 0.1$ HV vs. NC, $^{\theta}P \leq 0.1$ HVI vs. NC.

5.4.3. Plasma glucose, triglycerides, cholesterol, and BUN

Overall, the effects of diet and the interaction of diet by time on plasma glucose were significant ($P < 0.01$; Fig. 5.3A). At baseline, the plasma glucose for pigs fed with NC, HI and HV were not different relative to PC, but HVI had a higher plasma glucose than PC (Fig. 5.3A). Compared to NC, HV and HI were not different while HVI had a higher plasma glucose. HVI had a higher plasma glucose than HI at baseline. At 2 hours post meal, relative to PC, NC and HI had a lower plasma glucose (Fig. 5.3A). Further, HI had a lower plasma glucose than HV. Comparing plasma glucose for baseline and post meal, PC had a greater plasma glucose at 2 hours post meal than the baseline. Plasma glucose of HVI and HI at 2-hour post meal was lower and tended to be lower than baseline, respectively (Fig. 5.3A). Compared to PC, HVI had a lower triglyceride, but NC, HV and HI were not different (Fig. 5.3B). In comparison with PC, all groups increased the AUC cholesterol (Fig. 5.3C). Relative to NC, HVI had a lower BUN (Fig. 5.3D).

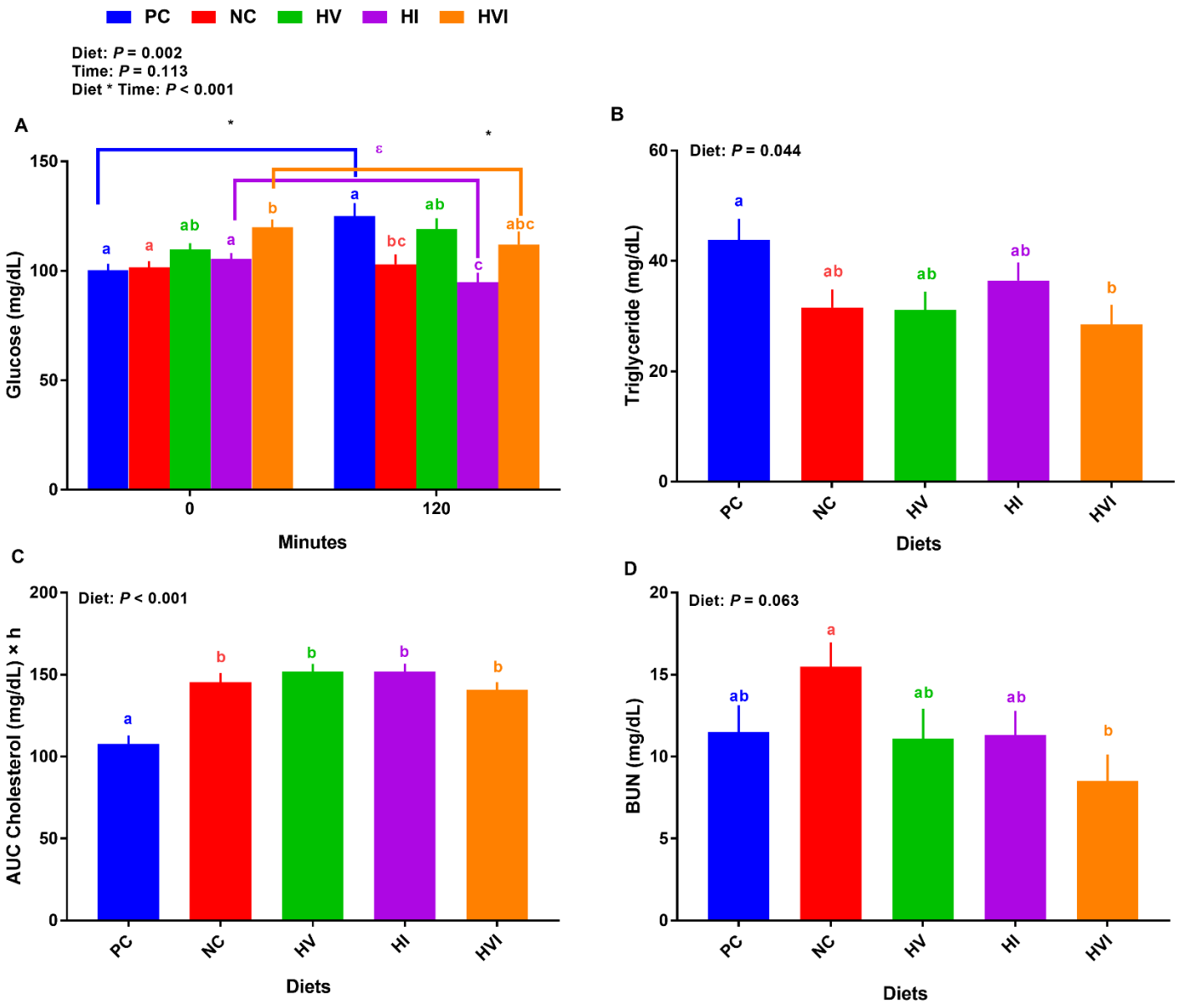


Figure 5.3. Plasma metabolite of nursery pigs fed with very low protein diets containing isoleucine (Ile), valine (Val) or mix of both above NRC levels.

(**A**) glucose concentration at baseline (0 min) and 120 min after meal, (**B**) triglyceride concentration, (**C**) area under the curve (AUC) for cholesterol, and (**D**) blood urea nitrogen (BUN). PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (*i.e.*, lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; HV: NC containing Val above NRC level; HI: NC containing

Ile above NRC level; HVI: NC containing both Val and Ile above NRC level. The values are means ± standard error of the mean. n=8. [a,b,c,ab,bc,abc] Among groups, the means with different superscript letter(s) are different ($P \leq 0.05$). *$P \leq 0.05$ 0 min vs. 120 min; [ε]$P \leq 0.1$ 0 min vs.120 min.

5.4.4. The mRNA abundance of key regulatory genes of lipid metabolism in liver and subcutaneous adipose tissue

Overall, the effect of diet on transcript of hepatic CPT1α, FAS, HSL, PPARα, and PGC1α was significant (Fig. 5.4C, D, F, G, H) and on mRNA abundance of hepatic SREBP-1 tended to be significant (Fig. 5.4I). No differences among treatments were detected for the mRNA abundance of hepatic ACC and CD36 (Fig. 5.4A and B). The mRNA abundance of CPT1α was lower in all groups compared to PC, but no differences among NC, HV, HI and HVI were detected (Fig. 5.4C). The transcript abundance of FAS was greater in NC and HV relative to PC, but that was not different for HI and HVI when compared to PC (Fig. 5.4D). The gene expression of HADH tended to be lower in HV compared to PC (Fig. 5.4E). In comparison with PC, the gene expression of hepatic HSL was reduced in NC, HV and HI, but not in HVI (Fig. 5.4F). Compared to PC, the hepatic transcript of PPARα was lower in all dietary treatments ($P < 0.05$; Fig. 5.4G). The transcript of PPARα was not different among NC, HV, HI and HVI. Relative to PC, the mRNA abundance of PGC1α tended to be lower in HV and HI ($P < 0.01$; Fig. 5.4H). The mRNA abundance of SREBP-1 tended to be higher in NC and HI in comparison with PC ($P < 0.1$; Fig. 5.4I).

Overall, the effect of diet on gene expression of LPL in subcutaneous adipose tissue was significant (Fig. 5A.3E). No differences among treatments were detected for the mRNA abundance of ACC, CD36, FAS, HADH, PPARα, PGC1α and SREBP-1 in subcutaneous adipose tissue (Fig. 5A.3A-D and 5A.3F-H). Relative to PC, transcript of LPL in adipose tissue was higher in NC and tended to be higher in HV, but not in HI and HVI (Fig. 5A.3E). The mRNA abundance of LPL tended to be lower in HI and HVI compared to NC and HV, respectively (Fig. 5A.3E).

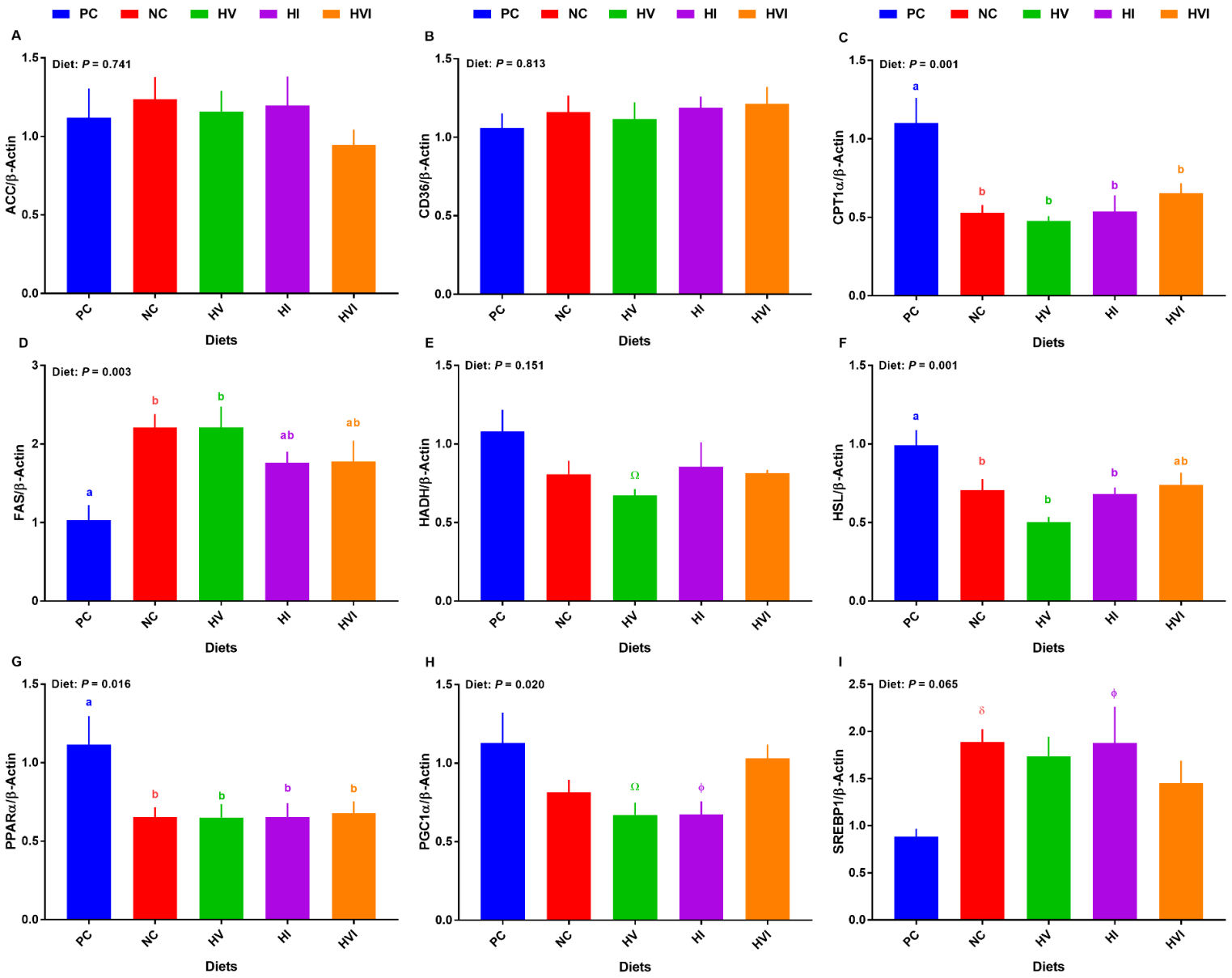

PC NC HV HI HVI
A
Diet: P = 0.741
ACC/β-Actin
B
Diet: P = 0.813
CD36/β-Actin
C
Diet: P = 0.001
CPT1α/β-Actin
D
Diet: P = 0.003
FAS/β-Actin
E
Diet: P = 0.151
HADH/β-Actin
F
Diet: P = 0.001
HSL/β-Actin
G
Diet: P = 0.016
PPARα/β-Actin
H
Diet: P = 0.020
PGC1α/β-Actin
I
Diet: P = 0.065
SREBP1/β-Actin
Diets

Figure 5.4. mRNA abundance of lipid metabolism markers in liver of nursery pigs fed with very low protein diets containing isoleucine (Ile), valine (Val) or mix of both above NRC levels.

(**A**) acetyl-CoA carboxylase alpha (ACC), (**B**) cluster of differentiation 36 molecule (CD36), (**C**) carnitine palmitoyltransferase I α (CPT1α), (**D**) fatty acid synthase (FAS), (**E**) hydroxyacyl-CoA dehydrogenase (HADH), (**F**) hormone-sensitive lipase (HSL), (**G**) peroxisome proliferator activated receptor alpha (PPARα), (**H**) PPARγ coactivator 1 alpha (PGC1α), (**I**) sterol regulatory element-binding protein 1 (SREBP-1). PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (*i.e.*, lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; HV: NC containing Val above NRC level; HI: NC containing Ile above NRC level; HVI: NC containing both Val and Ile above NRC level. The values are means ± standard error of the mean. n= 8. ^{a,b,ab} Among groups, the means with different superscript letter(s) are different ($P \leq 0.05$). $^{\delta}$ $P \leq 0.1$ PC vs. NC, $^{\Omega}$ $P \leq 0.1$ HV vs. PC, $^{\phi}$ $P \leq 0.1$ HI vs. PC.

5.4.5. The protein abundance of key regulatory genes of urea cycle enzymes in the liver and kidney

Overall, the effect of diet on protein expression of hepatic CPS-1α, OTC, and ASL was significant (Fig. 5.5A, B, E). Relative to PC, the protein abundance of CPS-1α and OTC were lower in all dietary treatments ($P < 0.01$; Fig. 5.5A and B), but those were not different among NC, HV, HI and HVI. No differences among treatments were detected for the protein abundance of ASS1, and ARG1 (Fig. 5.5C and D). Compared to PC, NC and HVI were not different in protein abundance of ASL, but that was lower in HV and HI (Fig. 5.5E). The protein abundance of ASL was not different among NC, HV, HI and HVI (Fig. 5.5E).

Overall, the effect of diet on protein expression of OTC in kidney tended to be significant ($P < 0.1$; Fig. 5.5F). In comparison with PC, the protein abundance of OTC tended to increase in HI (Fig. 5.5F). No differences among treatments were detected for the protein abundance of ASS1, ARG1 and ASL in kidney (Fig. 5.5 G-I).

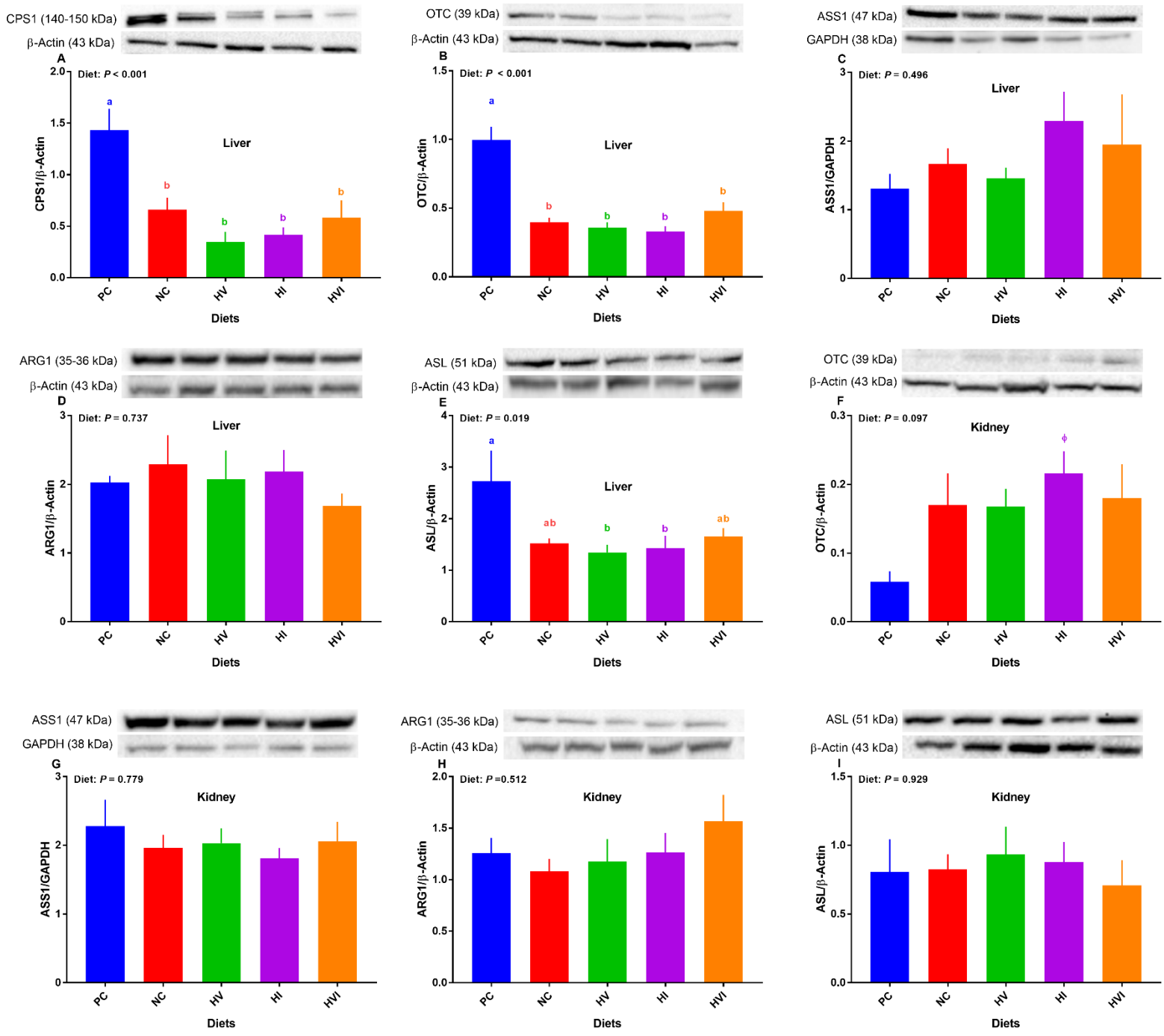


Figure 5.5. Relative protein abundance of urea cycle enzymes in liver (A-E) and kidney (F-I) of nursery pigs fed with very low protein diets containing isoleucine (Ile), valine (Val) or mix of both above NRC levels.

(**A**) carbamoyl phosphate synthetase I (CPS1) (**A**) ornithine transcarbamylase (OTC), (**B** and **F**) argininosuccinate synthase I (ASS1) (**C** and **G**), arginase I (ARG1) (**D** and **H**), argininosuccinate lyase (ASL) (**E** and **I**). GAPDH and β-actin were selected as a loading control. PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (i.e., lysine, methionine, threonine and tryptophan) at NRC (2012) levels; HV: NC containing Val above NRC level; HI: NC containing Ile above NRC level; HVI: NC containing both Val and Ile above NRC level. The values are means ± standard error of the mean. [a,b,ab] Among groups, the means with different superscript letter(s) are different ($P \leq 0.05$). n= 8. [ϕ] $P \leq 0.1$ HI vs. PC

5.5. Discussion

Supplementation of BCAA or mixture of Ile and Val not only decrease the negative impact of VLP diets on growth of pigs, but also reduce BUN, and influence the lipid metabolism [9-11, 33]. Little is understood on the mechanisms by which BCAA regulate the N utilization and lipid metabolism in pigs. The objective of current study was to assess the effect of dietary Ile and Val or their combination on gene or protein expression of key rate limiting enzymes involved in ureagenesis and fat metabolism (lipolysis and lipogenesis) in target tissues of pigs offered with VLP diets. Here we showed that: 1) HVI partially improved the BW and ADG and completely recovered the ADFI of pigs fed with VLP diets, 2) HVI increased the thermal radiation which is likely due to AA imbalance that partly contributes to a partial, but not complete recovery of BW in this group, 3) while HVI had a higher basal blood glucose, it showed a better glucose clearance than PC after a meal test, 4) HVI had a lower blood triglyceride than PC and BUN than NC suggestive of the role of Val and Ile on regulation of lipid metabolism and N utilization, 5) pigs fed with NC and HV seem to promote lipogenesis with increasing the mRNA abundance of hepatic FAS and SREBP1 and adipose LPL, but reducing transcript of hepatic HSL and PGC1α, 6) HVI seems to reduce the increased rate of lipogenesis in above mentioned groups through normalizing the transcript of hepatic FAS, SREBP1, HSL and PGC1α and LPL in adipose tissue, 7) all low protein groups (i.e. NC, HV, HI and HVI) reduced the ureagenesis through decreasing the protein expression of rate-limiting enzymes of ureagenesis, CPS1, OTC and ASL, in liver. Overall, a combination of dietary Val and Ile improved the growth, FI, and glucose clearance and decreased the rate of lipogenesis induced by VLP diets.

Feeding pigs with VLP diet supplemented with first four limiting AA decreased the BW and ADG, which is consistent with previous research [5-8]. In line with other studies [19, 65, 66], adding a combination of Val and Ile improved the growth and FI of pigs fed with VLP diets. In particular, HVI completely recovered the FI of VLP group to the levels seen in PC pigs. This is in parallel

with our recent data showing that supplementation of a combination of Val and Ile at NRC (2012) [51] level recovered the FI of pigs fed with VLP diets [11]. Improved FI in HVI group could be explained by changes in expression of peptides involved in FI regulation. Previously, we and others showed that the transcript of orexigenic neuropeptide Y and agouti-related protein was increased and that for anorexigenic proopiomelanocortin, melanocortin-4-receptor, and cocaine- and amphetamine regulated transcript was decreased in hypothalamus when BCAA or Val were added to low protein diets [14, 33, 67, 68].

While HVI completely recovered the FI, that showed only a partial improvement in BW and ADG. The lack of complete recovery in growth of HVI group might be attributed to a higher thermal radiation and energy loss in this group, which is consistent with our previous data [11]. The higher thermal radiation in HVI group is likely due to AA imbalances caused by a higher ratio of dietary Val and Ile to Lys and other AA. The AA imbalances in HVI group may not only influence the growth rate through reducing the availability of energy, but also may alter the efficiency of nutrients utilization [69]. The mechanisms by which the AA imbalances are sensed and possibly increase the energy expenditure [70] are not fully elucidated and further research is required. The first step of BCAA degradation is catabolized by a common enzyme, branched-chain α-ketoacid dehydrogenase and BCAA share the same transport system with other AA. Whether shared catabolic pathways and transport system among BCAA and other AA reduce the supply of Leu and essential AA in HVI group and whether that contributes to a higher thermal radiation in this group remains to be determined.

HVI pigs appeared to have a better glucose clearance than those fed with standard protein diets. Similarly, others showed that individual intrahypothalamic infusion of Ile and Val in rats reduced the postprandial blood glucose by lowering the hepatic glucose production [71]. In the current study, HI improved the glucose clearance after the meal test. This was in line with previous studies showing that intragastric infusion of Ile and Leu in healthy males [72, 73] or oral administration of

Ile in rats [74-76] and healthy humans [77], but not Val decreased the postprandial blood glucose. This is while intravenous infusion of Val in healthy humans [78] or its oral administration in rats [75] either marginally decreased or raised blood glucose concentration, respectively. Therefore, in our study the improved glucose tolerance in HVI group seems to be linked with the role of Ile in reducing blood glucose concentration. The hypoglycemic effects of Ile have been attributed to reduced hepatic gluconeogenesis and increased glucose uptake in skeletal muscle [74, 76].

Little is understood on the role of Ile and Val on lipid metabolism in pigs under protein restriction. Here we showed that NC and HV induced lipogenesis through increasing the gene expressions of FAS and SREBP1 in liver and LPL in adipose tissue and reducing transcript of hepatic HSL and PGC1α. Similar to our data, others showed that low protein diet reduce the hepatic expression of CPT1α in laying hens [79], but high protein diets increase hepatic mRNA expression of CPT1α in mice [80]. Further, here we showed that all low protein groups increased the blood cholesterol, which is in line with our and other previous studies showing that by reducing the level of dietary protein, plasma cholesterol increases in nursery pigs [7, 81]. In the present study, HVI seems to mitigate the rate of lipogenesis through normalizing the transcript of hepatic FAS, SREBP1, HSL and PGC1α and LPL in adipose tissue. HVI also reduced the blood triglyceride concentration. Likewise, we and others previously showed that BCAA supplementation decreases the blood triglycerides concentration in pigs and obese rats [33, 82]. The inhibitory role of HVI on lipogenesis could be due to different effects of Ile and Val on fat metabolism. While HV seems to have more positive effects on lipogenesis via decreasing the transcript of hepatic HADH, HSL and PGC1α, HI appears to reduce the lipogenesis by normalizing the hepatic FAS and LPL in adipose. Ile has been previously shown to stimulates lipolysis and reduces fat deposition, white adipose tissue mass and TG concentration in muscle and liver in mice [35]. Supplementation of Ile in drinking water of obese mice [36] and in the diet of broilers [83] has shown to reduce the white adipose tissue mass and body weight and the serum triglycerides, but Val supplementation increased serum TG in high

fat diet fed mice [38]. Therefore, the protective effects of HVI on lipogenesis might be explained by the buffering effect of Ile on Val-induced lipogenesis. Others showed that BCAA-supplemented mice had less weight, adipose tissue and hepatic TG content and downregulated the lipogenic enzymes in liver compared to their control counterparts [34, 37].

Branched-chain AA improve N utilization possibly through multiple mechanisms including increasing the activity and/or secretion rate of proteolytic enzymes [23-26]and expression of AA transporters in gut [27-29] and providing N for endogenous AA synthesis [30-32]. Little information is available whether BCAA and in particular Ile and Val alone or in combination affect the N balance through regulation of ureagenesis in liver and kidney. HVI had a lower BUN than NC in the present study. This is in parallel with previous studies showing that VLP diets supplemented with BCAA [9, 10] and mixture of Ile and Val [11] or standard protein diets supplemented with Val [12-14] and Ile [15, 84] had less BUN than un-supplemented pigs. A lower BUN in HVI group is suggestive of an improvement in N retention [16, 17]. Previous studies have indicated that BCAA improve the efficiency of AA and N utilization in pigs [18, 19, 85], and humans [20-22]. Although HVI had a lower BUN than NC, there were no differences in protein abundance of urea cycle rate limiting enzymes in liver and kidney between these two groups. Whether a higher BUN of NC versus HVI downregulates the urea cycle enzymes through a negative feedback mechanism as previously suggested [86] remains to be determined. Further, all low protein groups (*i.e.*, NC, HV, HI and HVI) decreased the ureagenesis through suppressing the protein expression of rate-limiting enzymes of ureagenesis, CPS1, OTC and ASL, in liver. The caveat of our study is that we measured the BUN in single time point at the end of study and it is not clear whether BUN concentration follows the same pattern in early stages of the study and those chronic changes have regulatory effects on urea cycle enzymes expression.

5.6. Conclusion

Supplementation of a mixture of dietary Val and Ile partially improved the growth and fully recovered the FI of pigs fed with VLP diets. A combination of Val and Ile added to VLP diets improved the glucose tolerance, reduced the rate of lipogenesis induced by VLP diets and decreased the BUN. Further research is required to better understand the regulatory role of BUN on ureagenesis under acute and chronic treatments with Ile and Val.

5.7. Appendices

Appendix Table 5A.1. The sequences [forward (F) and reveres (R)], location on template, amplicon size (bp), and GenBank accession numbers for primers used for reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR)

Genes[1]	Sequence (5' → 3')	Location on template	Amplicon length (bp)	GenBank accession no.	Reference
FAS	**F:** CTGCTGAAGCCTAACTCCTCG **R:** TTGCTCCTTGGAACCGTCTG	584 - 604 771 - 790	207	NM_001099930.1	Chen et al., 2014
ACC	**F:** ATGTTTCGGCAGTCCCTGAT **R:** TGTGGACCAGCTGACCTTGA	4870 - 4889 4983 - 5002	133	NM_001114269.1	Chen et al., 2014
HSL	**F:** GCTCCCATCGTCAAGAATC **R:** TAAAGCGAATGCGGTCC	2043 - 2061 2291 - 2307	265	NM_214315.3	Chen et al., 2014
PPARα	**F:** CATCCTCGCGGGAAAGG **R:** GGCCATACACAGTGTCTCCATGT	722 - 738 769 - 791	70	NM_001044526.1	Duran-Montgé et al., 2009
SREBP-1	**F**: CGGACGGCTCACAATGC **R**: GACGGCGGATTTATTCAGCTT	986 - 1002 1079 - 1099	114	NM_214157.1	Duran-Montgé et al., 2009
HADH	**F:** GCCATCGTGGAGAACCTGAA **R:** GAAATGGAGCCCGGCAAATC	461 - 480 600 - 619	159	NM_214331.1	Zuo et al., 2019
PGC1α	**F:** GATGTGTCGCCTTCTTGTTC **R:** CATCCTTTGGGGTCTTTGAG	1629 - 1648 1702 - 1721	93	NM_213963.2	Kang et al., 2018
LPL	**F:** CCCTATACAAGAGGGAACCGGAT **R:** CCGCCATCCAGTCGATAAACGT	448 - 470 564 - 580	138	NM_214286.1	Espinosa et al., 2020
CD36	**F:** CTGGTGCTGTCATTGGAGCAGT **R:** CTGTCTGTAAACTTCCGTGCCTGTT	443 - 464 579 - 603	161	NM_001044622.1	Espinosa et al., 2020
CPT1α	**F:** CAAGATGGGCATGAACGCTG **R:** TGGAATGTTGGGGTTGGTGT	1406 – 1425 1531 - 1550	145	NM_001129805.1	Zhou et al., 2017
β-Actin	**F:** CTGCGGCATCCACGAAACT **R:** AGGGCCGTGATCTCCTTCTG	944 - 962 1071 - 1090	147	XM_003124280.5	Yin et al., 2015

[1]FAS = fatty acid synthase; ACC = acetyl-CoA carboxylase; HSL = Hormone-sensitive lipase; PPARα = Peroxisome proliferator activated receptor alpha; SREBP-1 = sterol regulatory element-binding protein 1; HADH = Hydroxyacyl-CoA dehydrogenase; PGC1α = PPARγ coactivator 1 alpha; LPL = lipoprotein lipase; CD36 = cluster of differentiation 36 molecule, CPT1α = carnitine palmitoyltransferase 1 α.

Appendix Table 5A.2. The host, dilution, and supplier of primary and secondary antibodies for immunoblotting.

Antibodies	Host	Dilution	Vendor
Anti carbamoyl phosphate synthetase 1 (CPS1)	Rabbit	1:1000	Abcam, Cambridge, MA, # ab45956
Anti ornithine carbamoyl transferase (OTC)	Rabbit	1:1000	Millipore Sigma, St. Louis, MO, # AV41766
Anti argininosuccinate synthase 1 (ASS1)	Mouse	1:1000	Abcam, Cambridge, MA, # ab124465
Anti arginase 1 (ARG 1)	Rabbit	1:1000	Proteintech, Rosemont, IL, # 16001-1-AP
Anti arginosuccinate lyase (ASL)	Rabbit	1:1000	Abcam, Cambridge, MA, # ab97370
Anti β-actin (C4) (HRP)	Mouse	1:1000	Santa Cruz Biotechnology, Dallas, TX, # sc-47778
Anti GAPDH (GT239) (HRP)	Mouse	1:5000	Thermo Scientific, Rockford, IL, # MA5-31457
Anti rabbit IgG H&L (HRP)	Goat	1:2000	Abcam, Cambridge, MA, # ab205718
Anti mouse IgG H&L (HRP)	Goat	1:5000	Abcam, Cambridge, MA, # ab205719

Appendix Table 5A.3. Weekly growth performance of nursery pigs fed with very low-protein diets containing isoleucine, valine mix of both above NRC levels

	Diets[1]						
Measurements[2]	**PC**	**NC**	**HV**	**HI**	**HVI**	**SEM[3]**	***P*-value**
BWG, kg							
Week 1	2.49^{a}	1.35^{b}	2.07^{a}	1.28^{b}	2.04$^{\gamma}$	0.10	< 0.01
Week 2	3.50^{a}	1.52^{b}	1.64^{b}	1.62^{b}	2.90$^{\gamma}$	0.16	< 0.01
Week 3	4.29^{a}	1.92^{b}	1.77^{b}	2.40^{b}	2.82$^{\varepsilon}$	0.19	< 0.01
Week 4	5.06^{a}	2.72^{bc}	2.47^{b}	2.59^{b}	4.04$^{ac\varepsilon\tau}$	0.24	< 0.01
Week 5	5.15^{a}	2.67^{b}	3.16^{b}	2.87^{b}	3.44^{b}	0.22	< 0.01
MFI, kg							
Week 1	0.39	0.28	0.38	0.30$^{\phi}$	0.38	0.01	0.02
Week 2	0.65^{a}	0.39^{c}	0.49^{bc}	0.46^{bc}	0.58^{ab}	0.02	< 0.01
Week 3	0.89^{a}	0.52^{c}	0.61^{bc}	0.61^{bc}	0.78^{ab}	0.03	< 0.01
Week 4	0.97^{a}	0.68^{b}	0.80^{ab}	0.69^{b}	0.90^{ab}	0.03	0.01
Week 5	1.16^{a}	0.71^{b}	0.84^{b}	0.67^{b}	0.93$^{\gamma\tau}$	0.04	< 0.01
CFI, kg							
Week1	2.74	2.18	2.67	2.09$^{\phi}$	2.66	0.08	0.02
Week 2	4.54^{a}	2.73^{c}	3.43^{bc}	3.24^{bc}	4.04^{ab}	0.15	< 0.01
Week 3	6.24^{a}	3.63^{c}	4.28^{bc}	4.27^{bc}	5.49^{ab}	0.23	< 0.01
Week 4	6.81^{a}	4.76^{b}	5.58^{ab}	4.86^{b}	6.29^{ab}	0.23	0.01
Week 5	8.11^{a}	4.96^{b}	5.88^{b}	4.72^{b}	6.52^{ab}	0.28	< 0.01
CPI, kg							
Week 1	0.53^{a}	0.29^{b}	0.36^{b}	0.29^{b}	0.36^{b}	0.02	< 0.01
Week 2	0.89^{a}	0.36^{c}	0.46^{bc}	0.44^{bc}	0.54^{b}	0.03	< 0.01
Week 3	1.20^{a}	0.45^{c}	0.54^{bc}	0.59^{bc}	0.69^{b}	0.05	< 0.01
Week 4	1.37^{a}	0.50^{c}	0.71$^{b\vartheta}$	0.67^{bc}	0.79^{b}	0.05	< 0.01
Week 5	1.56^{a}	0.56^{b}	0.74^{bc}	0.65^{bc}	0.80$^{c\theta}$	0.07	< 0.01
G:F, kg/kg							
Week 1	0.89	0.62	0.78	0.67	0.80	0.03	0.09
Week 2	0.77^{a}	0.53^{bc}	0.49^{c}	0.50^{c}	0.68$^{a\theta}$	0.03	< 0.01
Week 3	0.69^{a}	0.54^{ab}	0.45^{b}	0.56^{ab}	0.49^{b}	0.02	< 0.01
Week 4	0.75	0.58	0.54	0.54	0.61	0.03	0.15
Week 5	0.64	0.53	0.53	0.64	0.53	0.03	0.49
G:P, kg/kg							
Week 1	4.57	4.54	5.76	4.79	5.88	0.24	0.22
Week 2	3.96^{ab}	3.89^{ab}	3.64^{b}	3.72^{b}	5.01$^{\theta}$	0.14	< 0.01
Week 3	3.57	4.42	3.45	4.09	3.90	0.14	0.17
Week 4	3.70	5.29	4.29	3.91	4.86	0.24	0.19
Week 5	3.33	4.46	4.30	4.61	4.28	0.22	0.32

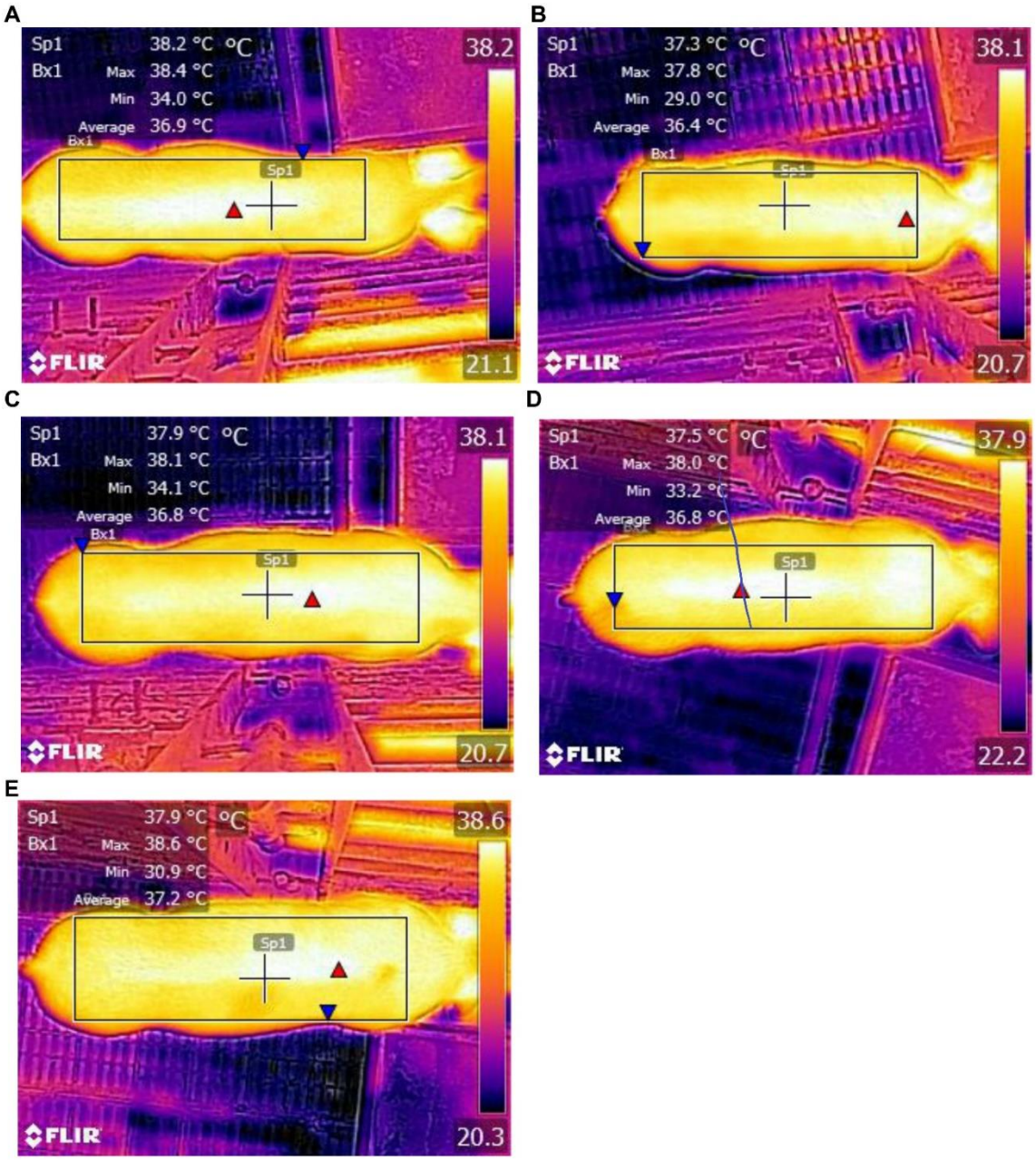
[1]PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (*i.e*., lysine, methionine, threonine and tryptophan) at NRC (2012) levels; HV: NC containing valine (Val) above NRC level; HI: NC containing isoleucine (Ile) above NRC level; HVI: NC containing both Val and Ile above NRC level.

The values are means, *n*=8. The *P*-values for the overall model effect for diet, week and diet × week for BWG were < 0.01, < 0.01 and < 0.01, for MFI were < 0.01, < 0.01 and < 0.01, for CFI were < 0.01, < 0.01 and < 0.01, for CPI were < 0.01, < 0.01 and < 0.01, for G:F were < 0.01, < 0.01 and 0.22, and for G:P were < 0.01, < 0.01 and 0.15 respectively.

[2]BWG: body weight gain; MFI: mean feed intake; CFI: cumulative feed intake; CPI: cumulative protein intake; G:F: gain:feed ratio; G:P: gain:protein ratio.

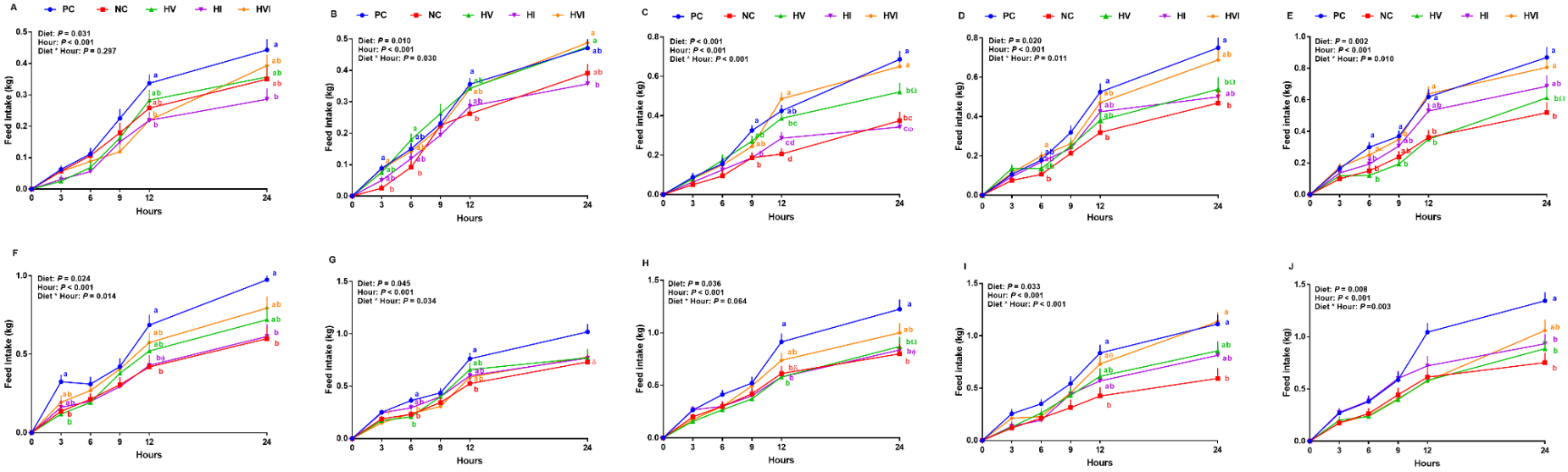
[3]SEM: standard error of the mean.

[a,b,c] Within each row, the values with different superscript letter(s) are different ($P \leq 0.05$). [γ] $P \leq 0.1$ HVI vs. PC, [θ] $P \leq 0.1$ HVI vs. NC, [ε] $P \leq 0.1$ HVI vs. HV, [τ] $P \leq 0.1$ HVI vs. HI, [ϕ] $P \leq 0.1$ HI vs. PC, [ϑ] $P \leq 0.1$ HV vs. NC.



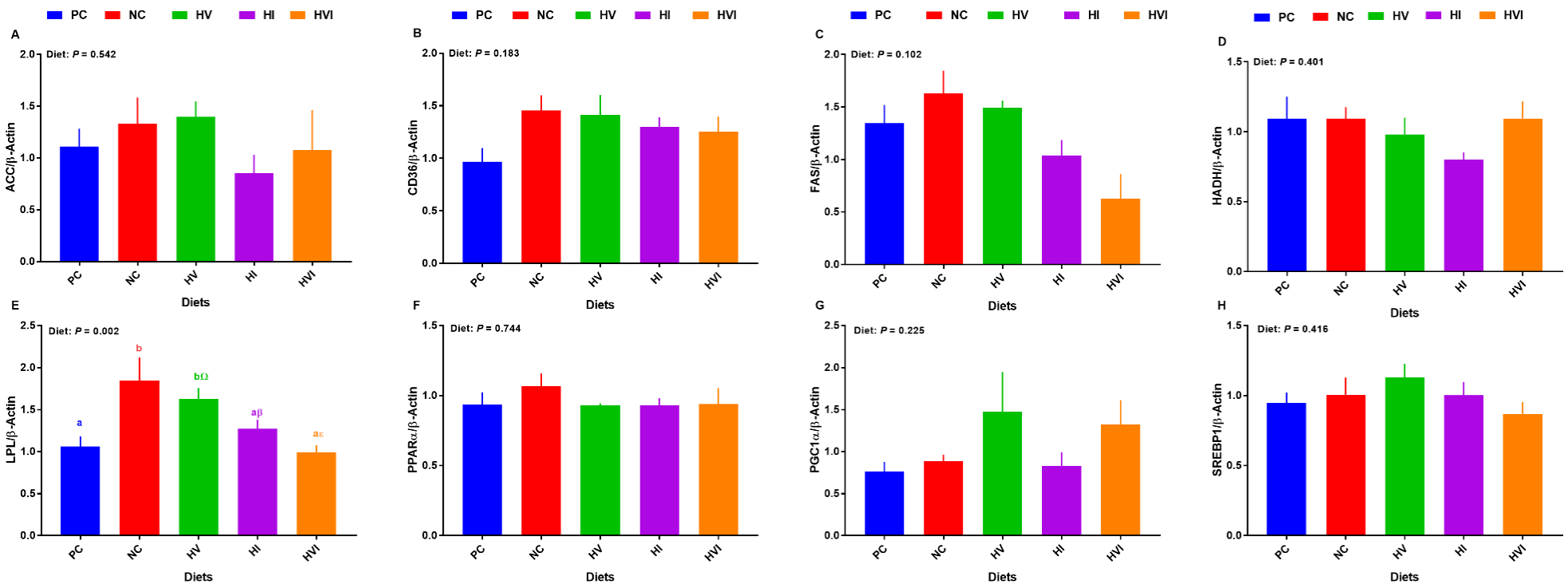
Appendix Figure 5A.1. Representative thermal images of dietary group.

(**A**) PC: positive control, standard protein diet, (**B**) NC: negative control, very low protein diet containing first four limiting amino acids (*i.e.*, lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; (**C**) HV: NC containing Val above NRC level; (**D**) HI: NC containing Ile above NRC level; (**E**) HVI: NC containing both Val and Ile above NRC level



Appendix Figure 5A.2. Feed intake of nursery pigs fed with very low protein diets containing isoleucine (Ile), valine (Val)or mix of both above NRC levels at (A) day 4, (B) day 7, (C) day 11 (D) day 14, (E) day 18, (F) day 21, (G) day 25, (H) day 28, (I) day 32, (J) day 35.

PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (*i.e.*, lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; HV: NC containing Val above NRC level; HI: NC containing Ile above NRC level; HVI: NC containing both Val and Ile above NRC level. The values are means ± standard error of the mean. n=8. ^{a,b,c,d,ab,bc,cd} the means with different superscript letter(s) at each time point are different ($P \leq 0.05$). $^{\delta}$ $P \leq 0.1$ PC vs. NC, $^{\Omega}$ $P \leq 0.1$ HV vs. PC, $^{\gamma}$ $P \leq 0.1$ HVI vs. PC, $^{\phi}$ $P \leq 0.1$ HI vs. PC, $^{\varepsilon}$ $P \leq 0.1$ HVI vs. HV, $^{\omega}$ $P \leq 0.1$ HV vs. HI



Appendix Figure 5A.3. mRNA abundance of lipid metabolism markers in subcutaneous adipose tissue of nursery pigs fed with very low protein diets containing isoleucine (Ile), valine (Val) or mix of both above NRC levels.

(**A**) acetyl-CoA carboxylase alpha (ACC), (**B**) cluster of differentiation 36 molecule (CD36), (**C**) fatty acid synthase (FAS), (**D**) hydroxyacyl-CoA dehydrogenase (HADH), (**E**) lipoprotein lipase (LPL), (**F**) peroxisome proliferator activated receptor alpha (PPARα), (**G**) PPARγ coactivator 1 alpha (PGC1α), (**H**) sterol regulatory element-binding protein 1 (SREBP-1). PC: positive control, standard protein diet; NC: negative control, very low protein diet containing first four limiting amino acids (i.e., lysine, methionine, threonine, and tryptophan) at NRC (2012) levels; HV: NC

containing Val above NRC level; HI: NC containing Ile above NRC level; HVI: NC containing both Val and Ile above NRC level. The values are means ± standard error of the mean. n= 8. [a,b,ab] Among groups, the means with different superscript letter(s) are different ($P \leq 0.05$). [Ω] $P \leq 0.1$ HV vs. PC, [β] $P \leq 0.1$ HI vs. NC, [ε] $P \leq 0.1$ HVI vs. HV.

5.8. References

1. Webb, J.; Broomfield, M.; Jones, S.; Donovan, B. Ammonia and odour emissions from UK pig farms and nitrogen leaching from outdoor pig production. A review. *Sci. Total Environ.* **2014**, *470*, 865–875. DOI: 10.1016/j.scitotenv.2013.09.091.

2. Deng, D.; Huang, R.; Li, T.; Wu, G.; Xie, M.; Tang, Z.; Kang, P.; Zhang, Y.; Fan, M.; Kong, X. Nitrogen balance in barrows fed low-protein diets supplemented with essential amino acids. *Livest. Sci.* **2007**, *109*, 220–223. DOI: 10.1016/j.livsci.2007.01.122.

3. He, L.; Wu, L.; Xu, Z.; Li, T.; Yao, K.; Cui, Z.; Yin, Y.; Wu, G. Low-protein diets affect ileal amino acid digestibility and gene expression of digestive enzymes in growing and finishing pigs. *Amino acids* **2016**, *48*, 21–30. DOI: 10.1007/s00726-015-2059-1.

4. Portejoie, S.; Dourmad, J.-Y.; Martinez, J.; Lebreton, Y. Effect of lowering dietary crude protein on nitrogen excretion, manure composition and ammonia emission from fattening pigs. *Livest. Prod. Sci.* **2004**, *91*, 45–55. DOI: 10.1016/j.livprodsci.2004.06.013.

5. Nyachoti, C.; Omogbenigun, F.; Rademacher, M.; Blank, G. Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. *J. Anim. Sci.* **2006**, *84*, 125–134. DOI: 10.2527/2006.841125x.

6. Wu, L.; Liao, P.; He, Q.; Tan, B.; Guo, F.; Tang, M.; Li, T. Chronic feeding with protein-restricted diets affect ileal amino acid digestibility and the expression of nutrient-sensing, hormone secretion, gastrointestinal digestive enzyme, and nutrient transporter genes in young weaned pigs. *Oncotarget* **2018**, *5*, DOI: Retrieved from https://www.oncotarget.com/article/24093/text/.

7. Spring, S.; Premathilake, H.; DeSilva, U.; Shili, C.; Carter, S.; Pezeshki, A. Low protein-high carbohydrate diets alter energy balance, gut microbiota composition and blood metabolomics profile in young pigs. *Sci. Rep.* **2020**, *10*, 1–15. DOI: 10.1038/s41598-020-60150-y.

8. Luo, Z.; Li, C.; Cheng, Y.; Hang, S.; Zhu, W. Effects of low dietary protein on the metabolites and microbial communities in the caecal digesta of piglets. *Arch. Anim. Nutr.* **2015**, *69*, 212–226. DOI: 10.1080/1745039X.2015.1034521.

9. Spring, S.; Premathilake, H.; Bradway, C.; Shili, C.; DeSilva, U.; Carter, S.; Pezeshki, A. Effect of very low-protein diets supplemented with branched-chain amino acids on energy balance, plasma metabolomics and fecal microbiome of pigs. *Sci. Rep.* **2020**, *10*, 1–16. DOI: 10.1038/s41598-020-72816-8.

10. Habibi, M.; Shili, C.N.; Sutton, J.; Goodarzi, P.; Pezeshki, A. Dietary branched-chain amino acids modulate the dynamics of calcium absorption and reabsorption in protein-restricted pigs. *J. Anim. Sci. Biotechnol.* **2022**, *13*, 1–16. DOI: 10.1186/s40104-021-00669-w.

11. Habibi, M.; Goodarzi, P.; Shili, C.N.; Sutton, J.; Wileman, C.M.; Kim, D.M.; Lin, D.; Pezeshki, A. A Mixture of Valine and Isoleucine Restores the Growth of Protein-Restricted Pigs Likely through Improved Gut Development, Hepatic IGF-1 Pathway, and Plasma Metabolomic Profile. *Int. J. Mol. Sci.* **2022**, *23*, 3300. DOI: 10.3390/ijms23063300.

12. Kwon, W.B.; Soto, J.A.; Stein, H.H. Effects on nitrogen balance and metabolism of branched-chain amino acids by growing pigs of supplementing isoleucine and valine to diets with adequate or excess concentrations of dietary leucine. *J. Anim. Sci.* **2020**, *98*, skaa346. DOI: 10.1093/jas/skaa346.

13. Liu, X.; Ma, W.; Zeng, X.; Xie, C.; Thacker, P.; Htoo, J.; Qiao, S. Estimation of the standardized ileal digestible valine to lysine ratio required for 25-to 120-kilogram pigs fed low crude protein diets supplemented with crystalline amino acids. *J. Anim. Sci.* **2015**, *93*, 4761–4773. DOI: 10.2527/jas.2015-9308.

14. Zhang, X.; Liu, X.; Jia, H.; He, P.; Mao, X.; Qiao, S.; Zeng, X. Valine supplementation in a reduced protein diet regulates growth performance partially through modulation of plasma amino acids profile, metabolic responses, endocrine, and neural factors in piglets. *J. Agric. Food Chem.* **2018**, *66*, 3161–3168. DOI: 10.1021/acs.jafc.8b01113.

15. Parr, T.; Kerr, B.; Baker, D. Isoleucine requirement of growing (25 to 45 kg) pigs. *J. Anim. Sci.* **2003**, *81*, 745–752. DOI: 10.2527/2003.813745x.

16. Lordelo, M.; Gaspar, A.; Le Bellego, L.; Freire, J. Isoleucine and valine supplementation of a low-protein corn-wheat-soybean meal-based diet for piglets: growth performance and nitrogen balance. *J. Anim. Sci.* **2008**, *86*, 2936–2941. DOI: 10.2527/jas.2007-0222.

17. Figueroa, J.; Lewis, A.; Miller, P.; Fischer, R.; Gómez, R.; Diedrichsen, R. Nitrogen metabolism and growth performance of gilts fed standard corn-soybean meal diets or low-crude protein, amino acid-supplemented diets. *J. Anim. Sci.* **2002**, *80*, 2911–2919. DOI: 10.2527/2002.80112911x.

18. Ren, M.; Zhang, S.; Zeng, X.; Liu, H.; Qiao, S. Branched-chain amino acids are beneficial to maintain growth performance and intestinal immune-related function in weaned piglets fed protein restricted diet. *Asian-Australasian J. Anim. Sci. s* **2015**, *28*, 1742. DOI: 10.5713/ajas.14.0131.

19. Ma, W.; Mao, P.; Fan, W.; Zhu, Y.; Guo, L. Valine and isoleucine supplementation improve performance and serum biochemical concentrations in growing gilts fed low-protein diets. *Canadian J. Anim. Sci.* **2019**, *99*, 921–928. DOI: 10.1139/cjas-2018-0169.

20. Echenique, M.; Bistrian, B.; Moldawer, L.; Palombo, J.; Miller, M.; Blackburn, G. Improvement in amino acid use in the critically ill patient with parenteral formulas enriched with branched chain amino acids. *Surg. Gynecol. Obstet.* **1984**, *159*, 233–241.

21. Wang, X.-Y.; Li, N.; Gu, J.; Li, W.-Q.; Li, J.-S. The effects of the formula of amino acids enriched BCAA on nutritional support in traumatic patients. *World J. Gastroenterol.* **2003**, *9*, 599. DOI: 10.3748/wjg.v9.i3.599.

22. Tamanna, N.; Mahmood, N. Emerging roles of branched-chain amino acid supplementation in human diseases. *Int Sch Res Notices* **2014**, *2014*, 235619. DOI: 10.1155/2014/235619.

23. Liu, K.; Liu, Y.; Liu, S.; Xu, M.; Yu, Z.; Wang, X.; Cao, Y.; Yao, J. Relationships between leucine and the pancreatic exocrine function for improving starch digestibility in ruminants. *J. Dairy Sci.* **2015**, *98*, 2576–2582. DOI: 10.3168/jds.2014-8404.

24. Zhao, J.; Liu, Y.; Jiang, J.; Wu, P.; Chen, G.; Jiang, W.; Li, S.; Tang, L.; Kuang, S.; Feng, L. Effects of dietary isoleucine on growth, the digestion and absorption capacity and gene expression in hepatopancreas and intestine of juvenile Jian carp (Cyprinus carpio var. Jian). *Aquaculture* **2012**, *368*, 117–128. DOI: 10.1016/j.aquaculture.2012.09.019.

25. Dong, M.; Feng, L.; Kuang, S.Y.; Liu, Y.; Jiang, J.; Hu, K.; Jiang, W.D.; Li, S.H.; Tang, L.; Zhou, X.Q. Growth, body composition, intestinal enzyme activities and microflora of juvenile Jian carp (Cyprinus carpio var. Jian) fed graded levels of dietary valine. *Aquac. Nutr.* **2013**, *19*, 1–14. DOI: 10.1111/j.1365-2095.2011.00926.x.

26. Guo, L.; Yao, J.; Zheng, C.; Tian, H.; Liu, Y.; Liu, S.; Cai, C.; Xu, X.; Cao, Y. Leucine regulates α-amylase and trypsin synthesis in dairy calf pancreatic tissue in vitro via the mammalian target of rapamycin signalling pathway. *Animal* **2019**, *13*, 1899–1906. DOI: 10.1017/S175173111800368.

27. Zhang, S.; Qiao, S.; Ren, M.; Zeng, X.; Ma, X.; Wu, Z.; Thacker, P.; Wu, G. Supplementation with branched-chain amino acids to a low-protein diet regulates intestinal

expression of amino acid and peptide transporters in weanling pigs. *Amino acids* **2013**, *45*, 1191–1205. DOI: 10.1007/s00726-013-1577-y.

28. Sun, Y.; Wu, Z.; Li, W.; Zhang, C.; Sun, K.; Ji, Y.; Wang, B.; Jiao, N.; He, B.; Wang, W. Dietary L-leucine supplementation enhances intestinal development in suckling piglets. *Amino Acids* **2015**, *47*, 1517–1525. DOI: 10.1007/s00726-015-1985-2.

29. Zhou, H.; Yu, B.; Gao, J.; Htoo, J.K.; Chen, D. Regulation of intestinal health by branched-chain amino acids. *Anim. Sci. J.* **2018**, *89*, 3–11. DOI: 10.1111/asj.12937.

30. Sciacovelli, M.; Dugourd, A.; Jimenez, L.V.; Yang, M.; Nikitopoulou, E.; Costa, A.S.; Tronci, L.; Caraffini, V.; Rodrigues, P.; Schmidt, C. Nitrogen partitioning between branched-chain amino acids and urea cycle enzymes sustains renal cancer progression. *BioRxiv* **2021**, DOI: 10.1101/2021.09.17.460635.

31. Gumus Balikcioglu, P.; Ramaker, M.E.; Mason, K.A.; Huffman, K.M.; Johnson, J.L.; Ilkayeva, O.; Muehlbauer, M.J.; Freemark, M.; Kraus, W.E. Branched-Chain Amino Acid Catabolism and Cardiopulmonary Function Following Acute Maximal Exercise Testing in Adolescents. *Front. cardiovasc. med.* **2021**, *8*, 721354. DOI: 10.3389/fcvm.2021.721354.

32. Wu, G. Amino acids: metabolism, functions, and nutrition. *Amino acids* **2009**, *37*, 1–17. DOI: 10.1007/s00726-009-0269-0.

33. Habibi, M.; Shili, C.; Sutton, J.; Goodarzi, P.; Maylem, E.R.; Spicer, L.; Pezeshki, A. Branched-chain amino acids partially recover the reduced growth of pigs fed with protein-restricted diets through both central and peripheral factors. *Anim. Nutr.* **2021**, *7*, 868–882. DOI: 10.1016/j.aninu.2021.02.002.

34. Arakawa, M.; Masaki, T.; Nishimura, J.; Seike, M.; Yoshimatsu, H. The effects of branched-chain amino acid granules on the accumulation of tissue triglycerides and uncoupling

proteins in diet-induced obese mice. *Endocr. J.* **2011**, *58*, 161–170. DOI: 10.1507/endocrj.k10e-221.

35. Nishimura, J.; Masaki, T.; Arakawa, M.; Seike, M.; Yoshimatsu, H. Isoleucine prevents the accumulation of tissue triglycerides and upregulates the expression of PPARα and uncoupling protein in diet-induced obese mice. *J. Nutr.* **2010**, *140*, 496–500. DOI: 10.3945/jn.109.108977.

36. Ma, Q.; Zhou, X.; Hu, L.; Chen, J.; Zhu, J.; Shan, A. Leucine and isoleucine have similar effects on reducing lipid accumulation, improving insulin sensitivity and increasing the browning of WAT in high-fat diet-induced obese mice. *Food function* **2020**, *11*, 2279–2290. DOI: 10.1039/C9FO03084K.

37. Zhang, F.; Zhao, S.; Yan, W.; Xia, Y.; Chen, X.; Wang, W.; Zhang, J.; Gao, C.; Peng, C.; Yan, F. Branched chain amino acids cause liver injury in obese/diabetic mice by promoting adipocyte lipolysis and inhibiting hepatic autophagy. *EBioMedicine* **2016**, *13*, 157–167. DOI: 10.1016/j.ebiom.2016.10.013.

38. Ma, Q.; Hu, L.; Zhu, J.; Chen, J.; Wang, Z.; Yue, Z.; Qiu, M.; Shan, A. Valine supplementation does not reduce lipid accumulation and improve insulin sensitivity in mice fed high-fat diet. *ACS omega* **2020**, *5*, 30937–30945. DOI: 10.1021/acsomega.0c03707.

39. Zhang, H.; Xiang, L.; Huo, M.; Wu, Y.; Yu, M.; Lau, C.W.; Tian, D.; Gou, L.; Huang, Y.; Luo, J.Y. Branched-chain amino acid supplementation impairs insulin sensitivity and promotes lipogenesis during exercise in diet-induced obese mice. *Obesity* **2022**, DOI: 10.1002/oby.23394.

40. Guo, F.; Cavener, D.R. The GCN2 eIF2α kinase regulates fatty-acid homeostasis in the liver during deprivation of an essential amino acid. *Cell metabolism* **2007**, *5*, 103–114. DOI: 10.1016/j.cmet.2007.01.001.

41. Cheng, Y.; Meng, Q.; Wang, C.; Li, H.; Huang, Z.; Chen, S.; Xiao, F.; Guo, F. Leucine deprivation decreases fat mass by stimulation of lipolysis in white adipose tissue and upregulation of uncoupling protein 1 (UCP1) in brown adipose tissue. *Diabetes* **2010**, *59,* 17–25. DOI: 10.2337/db09-0929.

42. Du, Y.; Meng, Q.; Zhang, Q.; Guo, F. Isoleucine or valine deprivation stimulates fat loss via increasing energy expenditure and regulating lipid metabolism in WAT. *Amino acids* **2012**, *43,* 725–734. DOI: 10.1007/s00726-011-1123-8.

43. Bai, J.; Greene, E.; Li, W.; Kidd, M.T.; Dridi, S. Branched-chain amino acids modulate the expression of hepatic fatty acid metabolism-related genes in female broiler chickens. *Molecular nutrition food research* **2015**, *59,* 1171–1181. DOI: 10.1002/mnfr.201400918.

44. McGarrah, R.W.; Zhang, G.-F.; Christopher, B.A.; Deleye, Y.; Walejko, J.M.; Page, S.; Ilkayeva, O.; White, P.J.; Newgard, C.B. Dietary branched-chain amino acid restriction alters fuel selection and reduces triglyceride stores in hearts of Zucker fatty rats. *American Journal of Physiology-Endocrinology Metabolism* **2020**, *318,* E216–E223. DOI: 10.1152/ajpendo.00334.2019.

45. Miller, E.; Ullrey, D. The pig as a model for human nutrition. *Annual review of nutrition* **1987**, *7,* 361–382. DOI: 10.1146/annurev.nu.07.070187.002045.

46. Baker, D.H. Animal models in nutrition research. *J. Nutr.* **2008**, *138,* 391–396. DOI: 10.1093/jn/138.2.391.

47. Ball, R.O.; Urschel, K.L.; Pencharz, P.B. Nutritional consequences of interspecies differences in arginine and lysine metabolism. *J. Nutr.* **2007**, *137,* 1626S–1641S. DOI: 10.1093/jn/137.6.1626S.

48. Riedijk, M.A.; Stoll, B.; Chacko, S.; Schierbeek, H.; Sunehag, A.L.; van Goudoever, J.B.; Burrin, D.G. Methionine transmethylation and transsulfuration in the piglet gastrointestinal tract. *Proc. Natl. Acad. Sci.* **2007**, *104*, 3408–3413. DOI: 10.1073/pnas.0607965104.

49. Bellinger, D.A.; Merricks, E.P.; Nichols, T.C. Swine models of type 2 diabetes mellitus: insulin resistance, glucose tolerance, and cardiovascular complications. *ILAR J.* **2006**, *47*, 243–258. DOI: 10.1093/ilar.47.3.243.

50. Harwood Jr, H.J.; Listrani, P.; Wagner, J.D.J.J.O.D.S.; technology. Nonhuman primates and other animal models in diabetes research. *J. Diabetes* **2012**, *6*, 503–514. DOI: 10.1177/1932296812006003.

51. NRC. *Nutrient Requirements of Swine: Eleventh Revised Edition*; The National Academies Press: Washington, DC, USA, 2012.

52. Nørgaard, J.; Fernández, J. Isoleucine and valine supplementation of crude protein-reduced diets for pigs aged 5–8 weeks. *Anim. Feed Sci. Technol.* **2009**, *154*, 248–253. DOI: 10.1016/j.anifeedsci.2009.08.010.

53. Goodarzi, P.; Habibi, M.; Roberts, K.; Sutton, J.; Shili, C.N.; Lin, D.; Pezeshki, A. Dietary tryptophan supplementation alters fat and glucose metabolism in a low-birthweight piglet model. *Nutrients* **2021**, *13*, 2561. DOI: 10.3390/nu13082561.

54. Shili, C.N.; Broomhead, J.N.; Spring, S.C.; Lanahan, M.B.; Pezeshki, A. A novel corn-expressed phytase improves daily weight gain, protein efficiency ratio and nutrients digestibility and alters fecal microbiota in pigs fed with very low protein diets. *Animals* **2020**, *10*, 1926. DOI: 10.3390/ani10101926.

55. Pezeshki, A.; Muench, G.; Chelikani, P. Expression of peptide YY, proglucagon, neuropeptide Y receptor Y2, and glucagon-like peptide-1 receptor in bovine peripheral tissues. *J. Dairy Sci.* **2012**, *95*, 5089–5094. DOI: 10.3168/jds.2011-5311.

56. Shili, C.N.; Habibi, M.; Sutton, J.; Barnes, J.; Burch-Konda, J.; Pezeshki, A. Effect of a phytogenic water additive on growth performance, blood metabolites and gene expression of amino acid transporters in nursery pigs fed with low-protein/high-carbohydrate diets. *Animals* **2021**, *11*, 555. DOI: 10.3390/ani11020555.

57. Zhou, X.; Wan, D.; Zhang, Y.; Zhang, Y.; Long, C.; Chen, S.; He, L.; Tan, B.; Wu, X.; Yin, Y. Diurnal variations in polyunsaturated fatty acid contents and expression of genes involved in their de novo synthesis in pigs. *Biochem. Biophys. Res. Commun.* **2017**, *483*, 430–434. DOI: 10.1016/j.bbrc.2016.12.126.

58. Espinosa, C.D.; Fry, R.S.; Kocher, M.E.; Stein, H.H. Effects of copper hydroxychloride on growth performance and abundance of genes involved in lipid metabolism of growing pigs. *J. Anim. Sci.* **2020**, *98*, skz369. DOI: 10.1093/jas/skz369.

59. Chen, G.; Zhang, J.; Zhang, Y.; Liao, P.; Li, T.; Chen, L.; Yin, Y.; Wang, J.; Wu, G. Oral MSG administration alters hepatic expression of genes for lipid and nitrogen metabolism in suckling piglets. *Amino Acids* **2014**, *46*, 245–250. DOI: 10.1007/s00726-013-1615-9.

60. Yin, J.; Liu, M.; Ren, W.; Duan, J.; Yang, G.; Zhao, Y.; Fang, R.; Chen, L.; Li, T.; Yin, Y. Effects of dietary supplementation with glutamate and aspartate on diquat-induced oxidative stress in piglets. *PloS one* **2015**, *10*, e0122893. DOI: 10.1371/journal.pone.0122893.

61. Zuo, F.; Gu, Q.; Li, S.; Wei, H.; Peng, J. Effects of different methionine sources on methionine metabolism in the IPEC-J2 cells. *Biomed Res. Int.* **2019**, *2019*, DOI: 10.1155/2019/5464906.

62. Duran-Montgé, P.; Theil, P.; Lauridsen, C.; Esteve-Garcia, E. Dietary fat source affects metabolism of fatty acids in pigs as evaluated by altered expression of lipogenic genes in liver and adipose tissues. *Animal* **2009**, *3*, 535–542. DOI: 10.1017/S1751731108003686.

63. Kang, P.; Liu, Y.; Zhu, H.; Zhang, J.; Shi, H.; Li, S.; Pi, D.; Leng, W.; Wang, X.; Wu, H. The effect of dietary asparagine supplementation on energy metabolism in liver of weaning pigs when challenged with lipopolysaccharide. *Asian-Australasian J. Anim. Sci. s* **2018**, *31*, 548.

64. Pezeshki, A.; Chelikani, P.K. Effects of Roux-en-Y gastric bypass and ileal transposition surgeries on glucose and lipid metabolism in skeletal muscle and liver. *Surg. Obes. Relat. Dis.* **2014**, *10*, 217–228. DOI: 10.1016/j.soard.2013.09.017.

65. Figueroa, J.; Lewis, A.; Miller, P.; Fischer, R.; Diedrichsen, R. Growth, carcass traits, and plasma amino acid concentrations of gilts fed low-protein diets supplemented with amino acids including histidine, isoleucine, and valine. *J. Anim. Sci.* **2003**, *81*, 1529–1537. DOI: 10.2527/2003.8161529x.

66. Russell, L.E.; Kerr, B.J.; Easter, R.A. Limiting amino acids in an 11% crude protein corn-soybean meal diet for growing pigs. *J. Anim. Sci.* **1987**, *65*, 1266–1272. DOI: 10.2527/jas1987.6551266x.

67. Zheng, L.; Wei, H.; Cheng, C.; Xiang, Q.; Pang, J.; Peng, J. Supplementation of branched-chain amino acids to a reduced-protein diet improves growth performance in piglets: involvement of increased feed intake and direct muscle growth-promoting effect. *Br. J. Nutr.* **2016**, *115*, 2236–2245. DOI: 10.1017/S0007114516000842.

68. Tian, M.; Heng, J.; Song, H.; Shi, K.; Lin, X.; Chen, F.; Guan, W.; Zhang, S. Dietary branched-chain amino acids regulate food intake partly through intestinal and hypothalamic amino

acid receptors in piglets. *J. Agric. Food Chem.* **2019**, *67,* 6809–6818. DOI: 10.1021/acs.jafc.9b02381.

69. Salmon, W.D. The significance of amino acid imbalance in nutrition. *Am. J. Clin. Nutr.* **1958**, *6,* 487–494. DOI: 10.1093/ajcn/6.5.487.

70. Xiao, F.; Guo, F. Impacts of essential amino acids on energy balance. *Mol. Metab.* **2021**, 101393. DOI: 10.1016/j.molmet.2021.101393.

71. Arrieta-Cruz, I.; Su, Y.; Gutiérrez-Juárez, R. Suppression of endogenous glucose production by isoleucine and valine and impact of diet composition. *Nutrients* **2016**, *8,* 79. DOI: 10.3390/nu8020079.

72. Elovaris, R.A.; Bitarafan, V.; Agah, S.; Ullrich, S.S.; Lange, K.; Horowitz, M.; Feinle-Bisset, C. Comparative Effects of the Branched-Chain Amino Acids, Leucine, Isoleucine and Valine, on Gastric Emptying, Plasma Glucose, C-Peptide and Glucagon in Healthy Men. *Nutrients* **2021**, *13,* 1613. DOI: 10.3390/nu13051613.

73. Ullrich, S.S.; Fitzgerald, P.C.; Schober, G.; Steinert, R.E.; Horowitz, M.; Feinle-Bisset, C. Intragastric administration of leucine or isoleucine lowers the blood glucose response to a mixed-nutrient drink by different mechanisms in healthy, lean volunteers. *Am. J. Clin. Nutr.* **2016**, *104,* 1274–1284. DOI: 10.3945/ajcn.116.140640.

74. Nishitani, S.; Takehana, K.; Fujitani, S.; onaka, I. Branched-chain amino acids improve glucose metabolism in rats with liver cirrhosis. *Am J Physiol Gastrointest Liver Physiol* **2005**, *288,* G1292–G1300. DOI: 10.1152/ajpgi.00510.2003.

75. Doi, M.; Yamaoka, I.; Fukunaga, T.; Nakayama, M. Isoleucine, a potent plasma glucose-lowering amino acid, stimulates glucose uptake in C2C12 myotubes. *Biochem. Biophys. Res. Commun.* **2003**, *312,* 1111–1117. DOI: 10.1016/j.bbrc.2003.11.039.

76. Doi, M.; Yamaoka, I.; Nakayama, M.; Sugahara, K.; Yoshizawa, F. Hypoglycemic effect of isoleucine involves increased muscle glucose uptake and whole body glucose oxidation and decreased hepatic gluconeogenesis. *Am. J. Physiol. Endocrinol. Metab.* **2007**, *292*, E1683–E1693. DOI: 10.1152/ajpendo.00609.2006.

77. Nuttall, F.Q.; Schweim, K.; Gannon, M.C. Effect of orally administered isoleucine with and without glucose on insulin, glucagon and glucose concentrations in non-diabetic subjects. *E Spen Eur E J Clin Nutr Metab* **2008**, *3*, e152–e158. DOI: 10.1016/j.eclnm.2008.05.001.

78. Floyd, J.; Fajans, S.S.; Conn, J.W.; Knopf, R.F.; Rull, J. Stimulation of insulin secretion by amino acids. *J. Clin. Investig.* **1966**, *45*, 1487–1502. DOI: 10.1172/JCI105456.

79. Wang, H.; Wang, X.; Zhao, J.; Jiao, H.; Lin, H. Low protein diet supplemented with crystalline amino acids suppressing appetite and apo-lipoprotein synthesis in laying hens. *Anim. Feed Sci. Technol.* **2020**, *266*, 114533. DOI: 10.1016/j.anifeedsci.2020.114533.

80. Garcia-Caraballo, S.C.; Comhair, T.M.; Verheyen, F.; Gaemers, I.; Schaap, F.G.; Houten, S.M.; Hakvoort, T.B.; Dejong, C.H.; Lamers, W.H.; Koehler, S.E. Prevention and reversal of hepatic steatosis with a high-protein diet in mice. *Biochimica et Biophysica Acta -Molecular Basis of Disease* **2013**, *1832*, 685–695. DOI: 10.1016/j.bbadis.2013.02.003.

81. Yu, D.; Zhu, W.; Hang, S. Effects of low-protein diet on the intestinal morphology, digestive enzyme activity, blood urea nitrogen, and gut microbiota and metabolites in weaned pigs. *Arch. Anim. Nutr.* **2019**, *73*, 287–305. DOI: 10.1080/1745039X.2019.1614849.

82. Bong, H.Y.; Kim, J.Y.; Jeong, H.I.; Moon, M.S.; Kim, J.; Kwon, O. Effects of corn gluten hydrolyzates, branched chain amino acids, and leucine on body weight reduction in obese rats induced by a high fat diet. *Nutr Res Pract* **2010**, *4*, 106–113. DOI: 10.4162/nrp.2010.4.2.106.

83. Lin, X.; Li, L.; Gou, Z.; Fan, Q.; Wang, Y.; Jiang, S. Reproductive performance, metabolism and oxidative stress profile in Chinese yellow-feathered broiler breeder hens fed multiple levels of isoleucine. *British Poultry Science* **2021**, *62*, 509–516. DOI: 10.1080/00071668.2021.1894322.

84. Richert, B.; Goodband, R.D.; Tokach, M.D.; Nelssen, J.L. Increasing valine, isoleucine, and total branched-chain amino acids for lactating sows. *J. Anim. Sci.* **1997**, *75*, 2117–2128. DOI: 10.2527/1997.7582117x.

85. Kwon, W.B.; Touchette, K.J.; Simongiovanni, A.; Syriopoulos, K.; Wessels, A.; Stein, H.H. Excess dietary leucine in diets for growing pigs reduces growth performance, biological value of protein, protein retention, and serotonin synthesis. *J. Anim. Sci.* **2019**, *97*, 4282–4292. DOI: 10.1093/jas/skz259.

86. Emmanuel, B. Autoregulation of urea cycle by urea in mammalian species. *Comp. Biochem. Physiol.* **1981**, *70*, 79–81. DOI: 10.1016/0300-9629(81)90398-4.

REFERENCES

Burton, A.S.; Stern, J.C.; Elsila, J.E.; Glavin, D.P.; Dworkin, J.P. Understanding prebiotic chemistry through the analysis of extraterrestrial amino acids and nucleobases in meteorites. Chem. Soc. Rev. 2012, 41, 5459– 5472. DOI: 10.1039/C2CS35109A

Kelly, B.; Pearce, E.L. Amino assets: how amino acids support immunity. Cell Metab. 2020, 32, 154– 175. DOI: 10.1016/j.cmet.2020.06.010

Wu, G. Amino acids: metabolism, functions, and nutrition. Amino acids. 2009, 37, 1– 17. DOI: 10.1007/s00726-009-0269-0

Doi, N.; Kakukawa, K.; Oishi, Y.; Yanagawa, H. High solubility of random-sequence proteins consisting of five kinds of primitive amino acids. Protein Eng. Des. Sel. 2005, 18, 279–284. DOI: 10.1093/protein/gzi034

Longo, L.M.; Tenorio, C.A.; Kumru, O.S.; Middaugh, C.R.; Blaber, M. A single aromatic core mutation converts a designed “primitive” protein from halophile to mesophile folding. Protein Sci. 2015, 24, 27–37. DOI: 10.1002/pro.2580

Lopez, M.J.; Mohiuddin, S.S. Biochemistry, essential amino acids; In: StatPearls Publishing: Treasure Island, USA, 2022.

Wu, G. Functional amino acids in nutrition and health. Amino acids. 2013, 45, 407–411. DOI: 10.1007/s00726-013-1500-6

Van Spronsen, F.J.; Hoeksma, M.; Reijngoud, D.J. Brain dysfunction in phenylketonuria: is phenylalanine toxicity the only possible cause? J. Inherit. Metab. Dis. 2009, 32, 46–51. DOI: 10.1007/s10545-008-0946-2

Andersen, A.E.; Avins, L. Lowering brain phenylalanine levels by giving other large neutral amino acids: a new experimental therapeutic approach to phenylketonuria. Arch. Neurol. 1976, 33, 684– 686. DOI: 10.1001/archneur.1976.00500100018008

Tackman, J.M., Tews, J.K. and Harper, A.E. Dietary disproportions of amino acids in the rat: effects on food intake, plasma and brain amino acids and brain serotonin. J. Nutr. 1990, 120, 521–533. DOI: 10.1093/jn/120.5.521

de Marte, M.L.; Enesco, H.E. Influence of low tryptophan diet on survival and organ growth in mice. Mech. Ageing Dev. 1986, 36, 161–171. DOI: 10.1016/0047-6374(86)90017-5

Eder, K.; Peganova, S.; Kluge, H. Studies on the tryptophan requirement of piglets. Arch. Anim. Nutr. 2001, 55, 281–279. DOI: 10.1080/17450390109386198

Shen, Y.B.; Voilqué, G.; Kim, J.D.; Odle, J.; Kim, S.W. Effects of increasing tryptophan intake on growth and physiological changes in nursery pigs. J. Anim. Sci. 2012, 90, 2264– 2275. DOI: 10.2527/jas.2011-4203

Segall, P.E.; Timiras, P.S. Patho-physiologic findings after chronic tryptophan deficiency in rats: a model for delayed growth and aging. Mech. Ageing Dev. 1976, 5, 109–124. DOI: 10.1016/0047-6374(76)90012-9

Moehn, S.; Pencharz, P.B.; Ball, R.O. Lessons learned regarding symptoms of tryptophan deficiency and excess from animal requirement studies. J. Nutr. 2012, 142, 2231S–2235S. DOI: 10.3945/jn.112.159061

Joint, F.A.O.; World Health Organization. Protein and amino acid requirements in human nutrition: report of a joint FAO/WHO/UNU expert consultation. W. H. O. 2007.

WHO, J. Protein and amino acid requirements in human nutrition. W. H. O. Tech. Rep. Series. 2007.

Comai, S.; Bertazzo, A.; Brughera, M.; Crotti, S. Tryptophan in health and disease. Adv. Clin. Chem. 2020, 95, 165–218. DOI: 10.1016/bs.acc.2019.08.005

Ren, M.; Zhang, S.H.; Zeng, X.F.; Liu, H.; Qiao, S.Y. Branched-chain amino acids are beneficial to maintain growth performance and intestinal immune-related function in weaned piglets fed protein restricted diet. Asian-australas. J. Anim. Sci. 2015, 28, 1742. DOI: 10.5713/ajas.14.0131

Solon-Biet, S.M.; Cogger, V.C., Pulpitel, T.; Wahl, D.; Clark, X.; Bagley, E.E.; Gregoriou, G.C.; Senior, A.M.; Wang, Q.P.; Brandon, A.E. Perks, R. Branched-chain amino acids impact health and lifespan indirectly via amino acid balance and appetite control. Nat. Metab. 2019, 1, 532–545. DOI: 10.1038/s42255-019-0059-2

Zhang, L.; Li, F.; Guo, Q.; Duan, Y.; Wang, W.; Yang, Y.; Yin, Y.; Gong, S.; Han, M.; Yin, Y. Different proportions of branched-chain amino acids modulate lipid metabolism in a finishing pig model. J. Agric. Food Chem. 2021, 69, 7037–7048. DOI: 10.1021/acs.jafc.1c02001

Mertz, E.T.; Beeson, W.M.; Jackson, H.D. Classification of essential amino acids for the weanling pig. Arch. Biochem. Biophys. 1952, 38, 121–128. DOI: 10.1016/0003-9861(52)90015-5

Gloaguen, M.; Le Floc'h, N.; Primot, Y.; Corrent, E.; van Milgen, J. Performance of piglets in response to the standardized ileal digestible phenylalanine and tyrosine supply in low-protein diets. Animal. 2014, 8, 1412– 1419. DOI: 10.1017/S1751731114001360

Fitzpatrick, P.F. Allosteric regulation of phenylalanine hydroxylase. Arch. Biochem. 2012, 519, 194– 201. DOI: 10.1016/j.abb.2011.09.012

Hsu, J.W.; Ball, R.O.; Evidence that phenylalanine may not provide the full needs for aromatic amino acids in children. Pediatr. Res. 2007, 61, 361– 365. DOI: 10.1203/pdr.0b013e318030d0db

Pencharz, P.B.; Hsu, J.W.; Ball, R.O. Aromatic amino acid requirements in healthy human subjects. J. Nutr. 2007, 137, 1576S– 1578S. DOI: 10.1093/jn/137.6.1576S

Akram, M.; Daniyal, M.; Ali, A.; Zainab, R.; Shah, S.M.A.; Munir, N.; Tahir, I.M. In Synucleins-Biochemistry and Role in Diseases; Surguchov, A., Eds.; IntechOpen, London., England, 2020; pp. 96. DOI: 10.5772/intechopen.73750

Rose, W.C. Nutrition Classics. Federation Proceedings 8 (2) 546-52, June 1949. Amino acid requirements of man. William C. Rose. Nutr. Rev. 1976, 34, 307–309. DOI: 10.1111/j.1753-4887.1976.tb05679.x

Onodera, R. Essentiality of histidine in ruminant and other animals including human beings. Asian-australas. J. Anim. Sci. 2003, 34, 445–454. DOI: 10.5713/ajas.2003.445

Holeček, M. Histidine in health and disease: metabolism, physiological importance, and use as a supplement. Nutrients 2020, 12, 848. DOI: 10.3390/nu12030848

Karau, A.; Grayson, I. Amino Acids in Human and Animal Nutrition. In Biotechnology of Food and Feed Additives. Advances in Biochemical Engineering/Biotechnology; Zorn, H.,

Czermak, P. Eds.; Springer: Berlin, Heidelberg, Germany, 2014; Volume 143, pp. 189–228. DOI: 10.1007/10_2014_269

Chen, Y.P., Cheng, Y.F., Li, X.H., Yang, W.L., Wen, C., Zhuang, S. and Zhou, Y.M. Effects of threonine supplementation on the growth performance, immunity, oxidative status, intestinal integrity, and barrier function of broilers at the early age. Poult. Sci.2017, 96, 405–413. DOI: 10.3382/ps/pew240

Estalkhzir, F.M.; Khojasteh, S.; Jafari, M. The effect of different levels of threonine on performance and carcass characteristics of broiler chickens. J. Nov. Appl. Sci. 2013, 2, 382–386.

Tang, Q.; Tan, P.; Ma, N.; Ma, X. Physiological functions of threonine in animals: beyond nutrition metabolism. Nutrients. 2013, 13, 2592. DOI: 10.3390/nu13082592

Wang, W.; Zeng, X.;Mao, X.; Wu, G.; Qiao, S. Optimal dietary true ileal digestible threonine for supporting the mucosal barrier in small intestine of weanling pigs. J. Nutr. 2010, 140, 981–986. DOI: 10.3945/jn.109.118497

Chen, Y.; Zhang, H.; Cheng, Y.; Li, Y.; Wen, C.; Zhou, Y. Dietary l-threonine supplementation attenuates lipopolysaccharide-induced inflammatory responses and intestinal barrier damage of broiler chickens at an early age. Br. J. Nutr. 2018, 119, 1254–1262. DOI: 10.1017/S0007114518000740

Cromwell, G.L. Identifying the limiting amino acids in complex and cereal grain-based diets to minimize nitrogen excretion. In Midwest Swine Nutrition Conference Nutrition Conference Proceedings. Indianapolis, IN, USA, The Ohio Univ. Press, Columbus, OH, USA; 2004, 69–83.

Dilger, R.N.; Baker, D.H. DL-Methionine is as efficacious as L-methionine, but modest L-cystine excesses are anorexigenic in sulfur amino acid-deficient purified and practical-type diets fed to chicks. Poult. Sci. 2007, 86, 2367–2374. DOI: 10.3382/ps.2007-00203

Shen, Y.B.; Weaver, A.C.; Kim, S.W. Effect of feed grade L-methionine on growth performance and gut health in nursery pigs compared with conventional DL-methionine. J. Anim. Sci. 2014, 92, 5530–5539. DOI: 10.2527/jas.2014-7830

Finkelstein, J.D. Methionine metabolism in mammals. J. Nutr. Biochem. 1990, 1, 228– 237. DOI: 10.1016/0955-2863(90)90070-2

Reddy, V.Y.; Desorchers, P.E.; Pizzo, S.V.; Gonias, S.L.; Sahakian, J.A.; Levine, R.L.; Weiss, S.J. Oxidative dissociation of human alpha 2-macroglobulin tetramers into dysfunctional dimers. J. Biol. Chem. 1994, 269, 4683–4691. DOI: 10.1016/S0021-9258(17)41830-8

Luo, S. and Levine, R.L. Methionine in proteins defends against oxidative stress. FASEB. J. 2009, 23, 464–472. DOI: 10.1096/fj.08-118414

Stoll, B.; Henry, J.; Reeds, P.J.; Yu, H.; Jahoor, F.; Burrin, D.G. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. J. Nutr. 1998, 128, 606–614. DOI: 10.1093/jn/128.3.606

Riedijk, M.A.; Stoll, B.; Chacko, S.; Schierbeek, H.; Sunehag, A.L., van Goudoever, J.B.; Burrin, D.G. Methionine transmethylation and transsulfuration in the piglet gastrointestinal tract. Proc. Natl. Acad. Sci. 2007, 104, 3408–3413. DOI: 10.1073/pnas.0607965104

Wu, G. Nutrition and Metabolism: Foundations for Animal Growth, Development, Reproduction, and Health. In Recent Advances in Animal Nutrition and Metabolism; Wu, G.

Eds.; Springer: Berlin, Heidelberg, Germany, 2022; Volume 1354, pp. 1–24. DOI: 10.1007/978-3-030-85686-1_1

Kiluk, M., Lewkowicz, J., Pawlak, D. Tankiewicz-Kwedlo, A. Crosstalk between Tryptophan Metabolism via Kynurenine Pathway and Carbohydrate Metabolism in the Context of Cardio-Metabolic Risk. J. Clin. Med. 2021, 10, 2484. DOI: 10.3390/jcm10112484

Ponter, A.A., Sève, B. and Morgan, L.M. Intragastric tryptophan reduces glycemia after glucose, possibly via glucose-mediated insulinotropic polypeptide, in early-weaned piglets. J. Nutr. 1994, 124, 259–267. DOI: 10.1093/jn/124.2.259

Zhang, L.; Ovchinnikova, O.; Jönsson, A.; Lundberg, A.M.; Berg, M.; Hansson, G.K.; Ketelhuth, D.F. The tryptophan metabolite 3-hydroxyanthranilic acid lowers plasma lipids and decreases atherosclerosis in hypercholesterolaemic mice. Eur. Heart J. 2012, 33, 2025–2034. DOI: 10.1093/eurheartj/ehs175

Nørgaard, J.V.; Pedersen, T.F.; Soumeh, E.A.; Blaabjerg, K.; Canibe, N.; Jensen, B.B.; Poulsen, H.D. Optimum standardized ileal digestible tryptophan to lysine ratio for pigs weighing 7–14 kg. Livest. Sci. 2015, 175, 90–95. DOI: 10.1016/j.livsci.2015.02.012

Sun, X.; Zemel, M.B. Leucine modulation of mitochondrial mass and oxygen consumption in skeletal muscle cells and adipocytes. Nutr. Metab. 2009, 6, 1–8. DOI: 10.1186/1743-7075-6-26

White, P.J.; Lapworth, A.L.; An, J.; Wang, L.; McGarrah, R.W.; Stevens, R.D.; Ilkayeva, O.; George, T.; Muehlbauer, M.J.; Bain, J.R.; Trimmer, J.K. Branched-chain amino acid restriction in Zucker-fatty rats improves muscle insulin sensitivity by enhancing efficiency of fatty acid

oxidation and acyl-glycine export. Mol. Metab. 2016, 5, 538–551. DOI: 10.1016/j.molmet.2016.04.006

Cammisotto, P.G., Gélinas, Y., Deshaies, Y. and Bukowiecki, L.J. Regulation of leptin secretion from white adipocytes by insulin, glycolytic substrates, and amino acids. Am. J. Physiol. Endocrinol. 2005, 289, E166– E171. DOI: 10.1152/ajpendo.00602.2004

Nuttall, F.Q.; Schweim, K.J.; Gannon, M.C. Effect of orally administered phenylalanine with and without glucose on insulin, glucagon and glucose concentrations. Horm. Metab. Res. 2006, 38, 518–523. DOI: 10.1055/s-2006-949523

Suzuki, R.; Sato, Y.; Fukaya, M.; Suzuki, D.; Yoshizawa, F.; Sato, Y. Energy metabolism profile of the effects of amino acid treatment on hepatocytes: phenylalanine and phenylpyruvate inhibit glycolysis of hepatocytes. Nutrition 2021, 82, 111042. DOI: 10.1016/j.nut.2020.111042

Wang, J.; Qiao, Y.; Sun, H.; Chang, H.; Zhao, H.; Zhang, S.; Shan, C. Decreased SLC27A5 Suppresses Lipid Synthesis and Tyrosine Metabolism to Activate the Cell Cycle in Hepatocellular Carcinoma. Horm. Metab. Res. 2022, 10, 234. DOI: 10.3390/biomedicines10020234

Xu, H.; Sakakibara, S.; Morifuji, M.; Salamatulla, Q.; Aoyama, Y. Excess dietary histidine decreases the liver copper level and serum alanine aminotransferase activity in Long-Evans Cinnamon rats. Br. J. Nutr. 2003, 90, 573–579. DOI: 10.1079/BJN2003939

Mong, M.C.; Chao, C.Y.; Yin, M.C. Histidine and carnosine alleviated hepatic steatosis in mice consumed high saturated fat diet. Eur. J. Pharmacol. 2011, 653, 82–88. DOI: 10.1016/j.ejphar.2010.12.001

DiNicolantonio, J.J.; McCarty, M.F.; OKeefe, J.H. Role of dietary histidine in the prevention of obesity and metabolic syndrome. Open Heart. 2018, 5, e000676. DOI: 10.1136/openhrt-2017-000676

Feng, R.N.; Niu, Y.C.; Sun, X.W.; Li, Q.; Zhao, C.; Wang, C.; Guo, F.C.; Sun, C.H.; Li, Y. Histidine supplementation improves insulin resistance through suppressed inflammation in obese women with the metabolic syndrome: a randomised controlled trial. Diabetologia 2013, 56, 985–994. DOI: 10.1007/s00125-013-2839-7

Ma, Q.; Zhou, X.; Sun, Y.; Hu, L.; Zhu, J.; Shao, C.; Meng, Q.; Shan, A. Threonine, but not lysine and methionine, reduces fat accumulation by regulating lipid metabolism in obese mice. J. Agric. Food Chem. 2020, 68, 4876–4883. DOI: 10.1021/acs.jafc.0c01023

Martínez, Y.; Li, X.; Liu, G.; Bin, P.; Yan, W.; Más, D.; Valdivié, M.; Hu, C.A.A.; Ren, W.; Yin, Y. The role of methionine on metabolism, oxidative stress, and diseases. Amino acids. 2017, 49, 2091–2098. DOI: 10.1007/s00726-017-2494-2

MacFarlane, N.G. Digestion and absorption. *Anaesth. Intensive Care Med.* **2018**, *19*, 125–127. DOI: 10.1016/j.mpaic.2018.01.001.

2. Gropper, S.S.; Smith, J.L., *Advanced nutrition and human metabolism*. 2012, Boston, MA: Cengage Learning.

3. Jackson, A.D.; McLaughlin, J. Digestion and absorption. *Surgery* **2009**, *27*, 231–236. DOI: 10.1016/j.mpsur.2009.03.003

Verger, R.; Rietsch, J.; Desnuelle, P. Effects of colipase on hydrolysis of monomolecular films by lipase. *J. Biol. Chem.* **1977**, *252*, 4319–4325. DOI: 10.1016/S0021-9258(17)40267-5

Brockerhoff, H. On the function of bile salts and proteins as cofactors of lipase. *J. Biol. Chem.* **1971**, *246*, 5828–5831. DOI: 0.1016/S0021-9258(18)61880-0

Hernell, O.; Staggers, J.E.; Carey, M.C. Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. *Biochemistry* **1990**, *29*, 2041–2056. DOI: 10.1021/bi00460a012

Hinsberger, A.; Sandhu, B. Digestion and absorption. *Current Paediatrics* **2004**, *14*, 605–611. DOI: 10.1016/j.cupe.2004.08.004

Yen, C.-L.E.; Nelson, D.W.; Yen, M.-I. Intestinal triacylglycerol synthesis in fat absorption and systemic energy metabolism. *J. Lipid Res.* **2015**, *56*, 489–501. DOI: 10.1194/jlr.R052902.

Kersten, S. Physiological regulation of lipoprotein lipase. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* **2014**, *1841*, 919–933. DOI: 10.1016/j.bbalip.2014.03.013

Davies, B.S.; Beigneux, A.P.; Barnes II, R.H.; Tu, Y.; Gin, P.; Weinstein, M.M.; Nobumori, C.; Nyrén, R.; Goldberg, I.; Olivecrona, G. GPIHBP1 is responsible for the entry of lipoprotein lipase into capillaries. *Cell Metab.* **2010**, *12*, 42–52. DOI: 10.1016/j.cmet.2010.04.016.

Beigneux, A.P.; Davies, B.S.; Gin, P.; Weinstein, M.M.; Farber, E.; Qiao, X.; Peale, F.; Bunting, S.; Walzem, R.L.; Wong, J.S. Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 plays a critical role in the lipolytic processing of chylomicrons. *Cell Metab.* **2007**, *5*, 279–291. DOI: 10.1016/j.cmet.2007.02.002.

Korn, E.D. Clearing factor, a heparin-activated lipoprotein lipase: I. Isolation and characterization of the enzyme from normal rat heart. *J. Biol. Chem.* **1955**, *215*, 1–14. DOI: 10.1016/S0021-9258(18)66011-9.

Kersten, S. Mechanisms of nutritional and hormonal regulation of lipogenesis. *EMBO reports* **2001**, *2*, 282–286. DOI: 10.1093/embo-reports/kve071

Horton, J.D.; Goldstein, J.L.; Brown, M.S. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Investig.* **2002**, *109*, 1125–1131. DOI: 10.1172/JCI15593

Schoonjans, K.; Staels, B.; Auwerx, J. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J. Lipid Res.* **1996**, *37*, 907–925. DOI: 10.1016/S0022-2275(20)42003-6

Lieberman, M.; Marks, A.D., *Marks' basic medical biochemistry: a clinical approach*. 2009: Lippincott Williams and Wilkins.

Binder, H.J.; Reuben, A. Nutrient digestion and absorption. *Medical physiology: a cellular molecular approach. Philadelphia: Saunders Elsevier* **2009**, 971.

Adeva-Andany, M.M.; Pérez-Felpete, N.; Fernández-Fernández, C.; Donapetry-García, C.; Pazos-García, C. Liver glucose metabolism in humans. *Biosci. Rep.* **2016**, *36*, DOI: 10.1042/BSR20160385.

Yeo, R.; Sawdon, M. Hormonal control of metabolism: regulation of plasma glucose. *Anaesth. Intensive Care Med.* **2013**, *14*, 296–300. DOI: 10.1016/j.mpaic.2013.04.009.

Giugliano, D.; Ceriello, A.; Esposito, K. Glucose metabolism and hyperglycemia. *Am. J. Clin. Nutr.* **2008**, *87*, 217S–222S.

Ang, M.; Linn, T. Comparison of the effects of slowly and rapidly absorbed carbohydrates on postprandial glucose metabolism in type 2 diabetes mellitus patients: a randomized trial. *Am. J. Clin. Nutr.* **2014**, *100*, 1059–1068.

Aronoff, S.L.; Berkowitz, K.; Shreiner, B.; Want, L. Glucose metabolism and regulation: beyond insulin and glucagon. *Diabetes Spectr.* **2004**, *17*, 183–190. DOI: 10.2337/diaspect.17.3.183.

Iynedjian, P.; Marie, S.; Gjinovci, A.; Genin, B.; Deng, S.-P.; Buhler, L.; Morel, P.; Mentha, G. Glucokinase and cytosolic phosphoenolpyruvate carboxykinase (GTP) in the human liver. Regulation of gene expression in cultured hepatocytes. *J. Clin. Investig.* **1995**, *95*, 1966–1973. DOI: 10.1172/JCI117880.

Woerle, H.J.; Meyer, C.; Dostou, J.M.; Gosmanov, N.R.; Islam, N.; Popa, E.; Wittlin, S.D.; Welle, S.L.; Gerich, J.E. Pathways for glucose disposal after meal ingestion in humans. *Am. J. Physiol. Endocrinol. Metab.* **2003**, *284*, E716–E725. DOI: 10.1152/ajpendo.00365.2002.

Chandramouli, V.; Ekberg, K.; Schumann, W.C.; Kalhan, S.C.; Wahren, J.; Landau, B.R. Quantifying gluconeogenesis during fasting. *Am. J. Physiol. Endocrinol. Metab.* **1997**, *273*, E1209–E1215. DOI: 10.1152/ajpendo.1997.273.6.E1209

Paksu, M.S.; Kalkan, G.; Asilioglu, N.; Paksu, S.; Dinler, G. Gluconeogenesis defect presenting with resistant hyperglycemia and acidosis mimicking diabetic ketoacidosis. *Pediatr. Emerg. Care* **2011**, *27*, 1180–1181. DOI: 10.1097/PEC.0b013e31823b412d.

Wu, G. Amino acids: metabolism, functions, and nutrition. *Amino Acids* **2009**, *37*, 1–17. DOI: 10.1007/s00726-009-0269-0.

Wu, G., *Amino acids: biochemistry and nutrition*. 2021: CRC Press. DOI: 10.1201/9781003092742

Takikawa, H.; Yamanaka, M. Mechanism of amino acid transport at the liver plasma membrane. *Nihon Rinsho* **1992**, *50*, 1475–1479.

Hou, Y.; Hu, S.; Li, X.; He, W.; Wu, G. Amino acid metabolism in the liver: nutritional and physiological significance. *Amino Acids in Nutrition Health* **2020**, 21–37. DOI: 10.1007/978-3-030-45328-2_2.

Wheatley, S.M.; El-Kadi, S.W.; Suryawan, A.; Boutry, C.; Orellana, R.A.; Nguyen, H.V.; Davis, S.R.; Davis, T.A. Protein synthesis in skeletal muscle of neonatal pigs is enhanced by administration of β-hydroxy-β-methylbutyrate. *Am. J Physiol. Endocrinol. Metab.* **2014**, *306,* E91–E99. DOI: 10.1152/ajpendo.00500.2013.

Brosnan, J.T. Interorgan amino acid transport and its regulation. *J. Nutr.* **2003**, *133,* 2068S–2072S. DOI: 10.1093/jn/133.6.2068S.

Gilbreath, K.R.; Bazer, F.W.; Satterfield, M.C.; Wu, G. Amino acid nutrition and reproductive performance in ruminants. In *Amino Acids in Nutrition and Health*, 2021, 43–61. DOI: 10.1007/978-3-030-54462-1_4.

Li, X.; Zheng, S.; Wu, G. Nutrition and functions of amino acids in fish. In *Amino Acids in Nutrition and Health*, 2021, 133–168. DOI: 10.1007/978-3-030-54462-1_8.

Rothman, S. How is the balance between protein synthesis and degradation achieved? *Theor. Biol. Medical Model.* **2010**, *7,* 1–11. DOI: 10.1186/1742-4682-7-25.

Gingras, A.-C.; Raught, B.; Sonenberg, N. eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Annu. Rev. Biochem.* **1999**, *68,* 913–963.

Wang, X.; Proud, C.G. The mTOR pathway in the control of protein synthesis. *Physiology* **2006**, *21,* 362–369. DOI: 10.1152/physiol.00024.2006.

Proud, C. Control of the elongation phase of protein synthesis. *Cold Spring Harbor Monogr. Ser*. **2000**, *39*, 719–740.

Bazer, F.W.; Song, G.; Kim, J.; Erikson, D.W.; Johnson, G.A.; Burghardt, R.C.; Gao, H.; Satterfield, M.C.; Spencer, T.E.; Wu, G. Mechanistic mammalian target of rapamycin (MTOR) cell signaling: effects of select nutrients and secreted phosphoprotein 1 on development of mammalian conceptuses. *Mol. Cell. Endocrinol.* **2012**, *354*, 22–33. DOI: 10.1016/j.mce.2011.08.026.

Wu, G.; Bazer, F.W.; Dai, Z.; Li, D.; Wang, J.; Wu, Z. Amino acid nutrition in animals: protein synthesis and beyond. *Annu Rev Anim Biosci* **2014**, *2*, 387–417. DOI: 10.1146/annurev-animal-022513-114113.

Morita, M.; Gravel, S.-P.; Hulea, L.; Larsson, O.; Pollak, M.; St-Pierre, J.; Topisirovic, I. mTOR coordinates protein synthesis, mitochondrial activity and proliferation. *Cell cycle* **2015**, *14*, 473–480. DOI: 10.4161/15384101.2014.991572.

Sarbassov, D.D.; Guertin, D.A.; Ali, S.M.; Sabatini, D.M. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* **2005**, *307*, 1098–1101. DOI: 10.1126/science.1106148.

Horman, S.; Browne, G.J.; Krause, U.; Patel, J.V.; Vertommen, D.; Bertrand, L.; Lavoinne, A.; Hue, L.; Proud, C.G.; Rider, M.H. Activation of AMP-activated protein kinase leads to the phosphorylation of elongation factor 2 and an inhibition of protein synthesis. *Curr. Biol.* **2002**, *12*, 1419–1423. DOI: 10.1016/S0960-9822(02)01077-1.

Browne, G.J.; Finn, S.G.; Proud, C.G. Stimulation of the AMP-activated protein kinase leads to activation of eukaryotic elongation factor 2 kinase and to its phosphorylation at a novel site, serine 398. *J. Biol. Chem.* **2004**, *279*, 12220–12231. DOI: 10.1074/jbc.M309773200.

Dimski, D.S. Ammonia metabolism and the urea cycle: function and clinical implications. *J. Vet. Intern. Med.* **1994**, *8*, 73–78. DOI: 10.1111/j.1939-1676.1994.tb03201.x.

Katunuma, N.; Okada, M.; Nishii, Y.J.A.i.e.r. Regulation of the urea cycle and TCA cycle by ammonia. **1966**, *4*, 317-335. DOI: 10.1016/0065-2571(66)90025-2

Song, X.; Teng, L.; Zhao, X. Re-examining the origin and functions of the urea cycle. **2022**, DOI: 10.21203/rs.3.rs-1532969/v1.

Jackson, M.J.; Beaudet, A.L.; O'Brien, W.E. Mammalian urea cycle enzymes. *Annu. Rev. Genet.* **1986**, *20*, 431–464. DOI: 10.1146/annurev.ge.20.120186.002243

Morris Jr, a.S.M. Regulation of enzymes of the urea cycle and arginine metabolism. *Annu. Rev. Nutr.* **2002**, *22*, 87–105. DOI: 10.1146/annurev.nutr.22.110801.140547.

Bigot, A.; Tchan, M.C.; Thoreau, B.; Blasco, H.; Maillot, F. Liver involvement in urea cycle disorders: a review of the literature. *J. Inherited Metab. Dis.* **2017**, *40*, 757–769. DOI: 10.1007/s10545-017-0088-5.

de Cima, S.; Polo, L.M.; Díez-Fernández, C.; Martínez, A.I.; Cervera, J.; Fita, I.; Rubio, V. Structure of human carbamoyl phosphate synthetase: deciphering the on/off switch of human ureagenesis. *J Scientific reports* **2015**, *5*, 1–15. DOI: 10.1038/srep16950.

Couchet, M.; Breuillard, C.; Corne, C.; Rendu, J.; Morio, B.; Schlattner, U.; Moinard, C. Ornithine Transcarbamylase–From Structure to Metabolism: An Update. *Front. Physiol.* **2021**, *12*, DOI: 10.3389/fphys.2021.748249.

Haines, R.J.; Pendleton, L.C.; Eichler, D.C. Argininosuccinate synthase: at the center of arginine metabolism. *Int. J. Biochem. Mol.* **2011**, *2*, 8.

Cao, Y.L.; Li, G.L.; Wang, K.T.; Zhang, H.Y.; Li, L.F. Crystallization and preliminary X-ray analysis of argininosuccinate lyase from Streptococcus mutans. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* **2011**, *67*, 682–684. DOI: 10.1107/S1744309111011377.

Caldwell, R.B.; Toque, H.A.; Narayanan, S.P.; Caldwell, R.W. Arginase: an old enzyme with new tricks. *Trends Pharmacol. Sci.* **2015**, *36*, 395–405. DOI: 10.1016/j.tips.2015.03.006.

Young, V.R.; El-Khoury, A.E.; Raguso, C.A.; Forslund, A.H.; Hambraeus, L. Rates of urea production and hydrolysis and leucine oxidation change linearly over widely varying protein intakes in healthy adults. *J. Nutr.* **2000**, *130*, 761–766. DOI: 10.1093/jn/130.4.761.

Wang, H.; Ran, J.; Jiang, T. Urea. *Urea Transporters* **2014**, 7–29. DOI: 10.1007/978-94-017-9343-8_2.

Emmanuel. Autoregulation of urea cycle by urea in mammalian species. *Comp. Biochem. Physiol.* **1981**, *70*, 79–81. DOI: 10.1016/0300-9629(81)90398-4.

Palego, L.; Betti, L.; Rossi, A.; Giannaccini, G. Tryptophan biochemistry: structural, nutritional, metabolic, and medical aspects in humans. *J. Amino Acids* **2016**, *2016*, DOI: 10.1155/2016/8952520.

de Jesus, A.J.; Allen, T.W. The role of tryptophan side chains in membrane protein anchoring and hydrophobic mismatch. *Biochim. Biophys. Acta - Bioenerg.* **2013**, *1828*, 864–876. DOI: 10.1016/j.bbamem.2012.09.009.

Grieco, P.; Cai, M.; Mayorov, A.V.; Trivedi, D.; Hruby, V.J. Structure–activity studies of new melanocortin peptides containing an aromatic amino acid at the N-terminal position. *Peptides* **2006**, *27,* 472–481. DOI: 10.1016/j.peptides.2005.01.032.

Mojsoska, B.; Jenssen, H. Peptides and peptidomimetics for antimicrobial drug design. *Pharmaceuticals* **2015**, *8,* 366–415. DOI: 10.3390/ph8030366.

Floc'h, L.; Otten, W.; Merlot, E. Tryptophan metabolism, from nutrition to potential therapeutic applications. *Amino Acids* **2011**, *41,* 1195–1205. DOI: 10.1007/s00726-010-0752-7.

Comai, S.; Bertazzo, A.; Brughera, M.; Crotti, S. Tryptophan in health and disease. *Adv. Clin. Chem.* **2020**, *95,* 165–218. DOI: 10.1016/bs.acc.2019.08.005.

Badawy, A.A. Kynurenine pathway of tryptophan metabolism: regulatory and functional aspects. *Int. J. Tryptophan Res.* **2017**, *10,* DOI: 10.1177/1178646917691938.

Bertazzo, A.; Ragazzi, E.; Biasiolo, M.; Costa, C.V.; Allegri, G. Enzyme activities involved in tryptophan metabolism along the kynurenine pathway in rabbits. *Biochim. Biophys. Acta - Biomembr. -General Sub.* **2001**, *1527,* 167–175. DOI: 10.1016/S0304-4165(01)00165-9.

Miller, C.L.; Llenos, I.C.; Dulay, J.R.; Barillo, M.M.; Yolken, R.H.; Weis, S. Expression of the kynurenine pathway enzyme tryptophan 2, 3-dioxygenase is increased in the frontal cortex of individuals with schizophrenia. *Neurobiol. Dis.* **2004**, *15,* 618–629. DOI: 10.1016/j.nbd.2003.12.015.

Fedorova, M.; Todorovsky, T.; Kuleva, N.; Hoffmann, R. Quantitative evaluation of tryptophan oxidation in actin and troponin I from skeletal muscles using a rat model of acute oxidative stress. *Proteomics* **2010**, *10,* 2692–2700. DOI: 10.1002/pmic.201000147.

Rexroth, S.; Poetsch, A.; Rögner, M.; Hamann, A.; Werner, A.; Osiewacz, H.D.; Schäfer, E.R.; Seelert, H.; Dencher, N.A. Reactive oxygen species target specific tryptophan site in the mitochondrial ATP synthase. *Biochim Biophys Acta-Bioenerg.* **2012**, *1817*, 381–387. DOI: 10.1016/j.bbabio.2011.11.006.

Ehrenshaft, M.; Deterding, L.J.; Mason, R.P.; Medicine. Tripping up Trp: Modification of protein tryptophan residues by reactive oxygen species, modes of detection, and biological consequences. *Free Radic. Biol.* **2015**, *89*, 220–228. DOI: 10.1016/j.freeradbiomed.2015.08.003.

Crotti, S.; Enzo, M.V.; Bedin, C.; Pucciarelli, S.; Maretto, I.; Del Bianco, P.; Traldi, P.; Tasciotti, E.; Ferrari, M.; Rizzolio, F. Clinical predictive circulating peptides in rectal cancer patients treated with neoadjuvant chemoradiotherapy. *J. Cell. Physiol.* **2015**, *230*, 1822–1828. DOI: 10.1002/jcp.24894.

Lucki, I. The spectrum of behaviors influenced by serotonin. *Biol. Psychiatry* **1998**, *44*, 151–162. DOI: 10.1016/S0006-3223(98)00139-5.

Comai, S.; Gobbi, G. CCNP Award Paper: Unveiling the role of melatonin MT2 receptors in sleep, anxiety and other neuropsychiatric diseases: a novel target in psychopharmacology. *J. Psychiatry. Neurosci.* **2014**, *39*, 6–21. DOI: 10.1503/jpn.130009.

Jockers, R.; Delagrange, P.; Dubocovich, M.L.; Markus, R.P.; Renault, N.; Tosini, G.; Cecon, E.; Zlotos, D.P. Update on melatonin receptors: IUPHAR Review 20. *Br. J. Pharmacol.* **2016**, *173*, 2702–2725. DOI: 10.1111/bph.13536.

Rogers, S.R.; Pesti, G.M. The influence of dietary tryptophan on broiler chick growth and lipid metabolism as mediated by dietary protein levels. *Poult. Sci.* **1990**, *69*, 746–756. DOI: 10.3382/ps.0690746.

Fears, R.; Murrellt, E.A. Tryptophan and the control of triglyceride and carbohydrate metabolism in the rat. *Br. J. Nutr.* **1980**, *43*, 349–356. DOI: 10.1079/BJN19800097.

Ruan, Z.; Yang, Y.; Zhou, Y.; Wen, Y.; Ding, S.; Liu, G.; Wu, X.; Liao, P.; Deng, Z.; Assaad, H. Metabolomic analysis of amino acid and energy metabolism in rats supplemented with chlorogenic acid. *Amino Acids* **2014**, *46*, 2219–2229. DOI: 10.1007/s00726-014-1762-7.

Eleftheriadis, T.; Pissas, G.; Sounidaki, M.; Tsogka, K.; Antoniadis, N.; Antoniadi, G.; Liakopoulos, V.; Stefanidis, I. Indoleamine 2, 3-dioxygenase, by degrading L-tryptophan, enhances carnitine palmitoyltransferase I activity and fatty acid oxidation, and exerts fatty acid-dependent effects in human alloreactive CD4+ T-cells. *Int. J. Mol. Med.* **2016**, *38*, 1605–1613. DOI: 10.3892/ijmm.2016.2750.

Sun, H.; Huang, F.-f.; Qu, S. Melatonin: a potential intervention for hepatic steatosis. *Lipids Health Dis.* **2015**, *14*, 1–6. DOI: 10.1186/s12944-015-0081-7.

Hedo, J.; Villanueva, M.L.; Marco, J. Elevation of plasma glucose and glucagon after tryptophan ingestion in man. *Metabolism* **1977**, *26*, 1131–1134. DOI: 10.1016/0026-0495(77)90040-3.

Inubushi, T.; Kamemura, N.; Oda, M.; SAkURAI, J.; NAkAYA, Y.; Harada, N.; Suenaga, M.; Matsunaga, Y.; Ishidoh, K.; Katunuma, N. L-tryptophan suppresses rise in blood glucose and preserves insulin secretion in type-2 diabetes mellitus rats. *J. Nutr. Sci. Vitaminol.* **2012**, *58*, 415–422. DOI: 10.3177/jnsv.58.415.

Sugimoto, Y.; Kimura, I.; Yamada, J.; Watanabe, Y.; Takeuchi, N.; Horisaka, K. The involvement of insulin in tryptamine-induced hypoglycemia in mice. *Life Sci.* **1991**, *48,* 1679–1683. DOI: 10.1016/0024-3205(91)90128-X.

83. Agil, A.; Rosado, I.; Ruiz, R.; Figueroa, A.; Zen, N.; Fernández-Vázquez, G. Melatonin improves glucose homeostasis in young Zucker diabetic fatty rats. *J. Pineal Res.* **2012**, *52,* 203–210. DOI: 10.1111/j.1600-079X.2011.00928.x.

Lima, F.B.; Machado, U.F.; Bartol, I.; Seraphim, P.M.; Sumida, D.H.; Moraes, S.M.; Hell, N.S.; Okamoto, M.M.; Saad, M.J.; Carvalho, C.R. Pinealectomy causes glucose intolerance and decreases adipose cell responsiveness to insulin in rats. *Am. J. Physiol. Endocrinol. Metab.* **1998**, E934–E941. DOI: 10.1152/ajpendo.1998.275.6.E934.

Diaz, B.; Blazquez, E. Effect of pinealectomy on plasma glucose, insulin and glucagon levels in the rat. *Horm. Metab. Res.* **1986**, *18,* 225–229.

Nogueira, T.C.; Lellis-Santos, C.; Jesus, D.S.; Taneda, M.; Rodrigues, S.C.; Amaral, F.G.; Lopes, A.M.S.; Cipolla-Neto, J.; Bordin, S.; Anhê, G.F. Absence of melatonin induces night-time hepatic insulin resistance and increased gluconeogenesis due to stimulation of nocturnal unfolded protein response. *Endocrinology* **2011**, *152,* 1253–1263. DOI: 10.1210/en.2010-1088.

Tresguerres, J.A.; Cuesta, S.; Kireev, R.A.; Garcia, C.; Acuña-Castroviejo, D.; Vara, E. Beneficial effect of melatonin treatment on age-related insulin resistance and on the development of type 2 diabetes. *Horm. Mol. Biol. Clin. Investig.* **2013**, *16,* 47–54. DOI: 10.1515/hmbci-2013-0041.

Tudhope, S.J.; Wang, C.-C.; Petrie, J.L.; Potts, L.; Malcomson, F.; Kieswich, J.; Yaqoob, M.M.; Arden, C.; Hampson, L.J.; Agius, L. A novel mechanism for regulating hepatic glycogen synthesis involving serotonin and cyclin-dependent kinase-5. *Diabetes* **2012**, *61*, 49–60. DOI: 10.2337/db11-0870.

Moore, M.C.; Kimura, K.; Shibata, H.; Honjoh, T.; Saito, M.; Everett, C.A.; Smith, M.S.; Cherrington, A.D. Portal 5-hydroxytryptophan infusion enhances glucose disposal in conscious dogs. *Am. J. Physiol. Endocrinol. Metab.* **2005**, *289*, E225–E231. DOI: 10.1152/ajpendo.00614.2004.

Hajduch, E.; Rencurel, F.; Balendran, A.; Batty, I.H.; Downes, C.P.; Hundal, H.S. Serotonin (5-hydroxytryptamine), a novel regulator of glucose transport in rat skeletal muscle. *J. Biol. Chem.* **1999**, *274*, 13563–13568. DOI: 10.1074/jbc.274.19.13563.

McDaniel, E.; Hundley, J.; Sebrell, W. Tryptophan-Niacin Metabolism in Alloxan Diabetic Rats: Two Figures. *J. Nutr.* **1956**, *59*, 407–423. DOI: 10.1093/jn/59.3.407.

Ray, P.D.; Foster, D.O.; Lardy, H.A. Paths of carbon in gluconeogenesis and lipogenesis: IV. Inhibition by L-tryptophan of hepatic gluconeogenesis at the level of phosphoenolpyruvate formation. *J. Biol. Chem.* **1966**, *241*, 3904–3908. DOI: 10.1016/S0021-9258(18)99792-9.

Foster, D.O.; Ray, P.D.; Lardy, H.A. A paradoxical in vivo effect of L-tryptophan on the phosphoenolpyruvate carboxykinase of rat liver. *Biochemistry* **1966**, *5*, 563–569.

Rippe, D.F.; Berry, L.J. Effect of endotoxin on the activation of phosphoenolpyruvate carboxykinase by tryptophan. *Infection Immunity* **1972**, *6*, 97–98.

Ardiansyah, S.; Shirakawa, H.; Inagawa, Y.; Koseki, T.; Komai, M. Regulation of blood pressure and glucose metabolism induced by L-tryptophan in stroke-prone spontaneously hypertensive rats. *Nutr. Metab.* **2011**, *8*, 1–7. DOI: 10.1186/1743-7075-8-45.

Ji, K.; Liang, H.; Chisomo-Kasiya, H.; Mokrani, A.; Ge, X.; Ren, M.; Liu, B. Effects of dietary tryptophan levels on growth performance, whole body composition and gene expression levels related to glycometabolism for juvenile blunt snout bream, Megalobrama amblycephala. *Aquacult. Nutr.* **2018**, *24*, 1474–1483. DOI: 10.1111/anu.12684.

Rubin, B.Y.; Anderson, S.L.; Xing, L.; Powell, R.J.; Tate, W. Interferon induces tryptophanyl-tRNA synthetase expression in human fibroblasts. *J. Biol. Chem.* **1991**, *266*, 24245–24248. DOI: 10.1016/S0021-9258(18)54219-8.

Jin, M. Unique roles of tryptophanyl-tRNA synthetase in immune control and its therapeutic implications. *Exp. Mol. Med.* **2019**, *51*, 1–10. DOI: 10.1038/s12276-018-0196-9.

Nørgaard, J.; Pedersen, T.; Soumeh, E.; Blaabjerg, K.; Canibe, N.; Jensen, B.; Poulsen, H. Optimum standardized ileal digestible tryptophan to lysine ratio for pigs weighing 7–14 kg. *Livest. Sci.* **2015**, *175*, 90–95. DOI: 10.1016/j.livsci.2015.02.012.

De Ridder, K.; Levesque, C.; Htoo, J.; De Lange, C. Immune system stimulation reduces the efficiency of tryptophan utilization for body protein deposition in growing pigs. *J. Anim. Sci.* **2012**, *90*, 3485–3491. DOI: 10.2527/jas.2011-4830.

Fürst, P.; Guarnieri, G.; Hultman, E. The effect of the administration of L-tryptophan on synthesis of urea and gluconeogenesis in man. *Scand. J. Clin. Lab. Invest.* **1971**, *27*, 183–191. DOI: 10.3109/00365517109080206.

Pátek, M. Branched-chain amino acids. In *Amino Acid Biosynthesis~ Pathways, Regulation and Metabolic Engineering*, 2007, 129–162.

Adeva, M.M.; Calviño, J.; Souto, G.; Donapetry, C. Insulin resistance and the metabolism of branched-chain amino acids in humans. *Amino Acids* **2012**, *43*, 171–181. DOI: 10.1007/s00726-011-1088-7.

Barnes, M.R.; Gray, I.C., *Bioinformatics for geneticists*. 2003, England: John Wiley and Sons.

Brosnan, J.T.; Brosnan, M.E. Branched-chain amino acids: enzyme and substrate regulation. *J. Nutr*. **2006**, *136*, 207S–211S. DOI: 10.1093/jn/136.1.207S.

Gu, C.; Mao, X.; Chen, D.; Yu, B.; Yang, Q. Isoleucine plays an important role for maintaining immune function. *Curr Protein Pept Sci* **2019**, *20*, 644–651. DOI: 10.2174/1389203720666190305163135.

Kainulainen, H.; Hulmi, J.J.; Kujala, U.M.J.E.; reviews, s.s. Potential role of branched-chain amino acid catabolism in regulating fat oxidation. **2013**, *41*, 194-200.

Zhang, S.; Zeng, X.; Ren, M.; Mao, X.; Qiao, S. Novel metabolic and physiological functions of branched chain amino acids: a review. *J. Anim. Sci. Biotechnol.* **2017**, *8*, 1–12. DOI: 10.1186/s40104-016-0139-z.

Sperringer, J.E.; Addington, A.; Hutson, S.M. Branched-chain amino acids and brain metabolism. *Neurochem. Res.* **2017**, *42*, 1697–1709. DOI: 10.1007/s11064-017-2261-5.

Kadowaki, T.; Yamauchi, T. Adiponectin and adiponectin receptors. *Endocr. Rev.* **2005**, *26*, 439–451. DOI: 10.1210/er.2005-0005.

Nishimura, J.; Masaki, T.; Arakawa, M.; Seike, M.; Yoshimatsu, H. Isoleucine prevents the accumulation of tissue triglycerides and upregulates the expression of PPARα and uncoupling protein in diet-induced obese mice. *J. Nutr.* **2010**, *140*, 496–500. DOI: 10.3945/jn.109.108977.

Feldmann, H.M.; Golozoubova, V.; Cannon, B.; Nedergaard, J. UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metab.* **2009**, *9*, 203–209. DOI: 10.1016/j.cmet.2008.12.014.

Masaki, T.; Yoshimatsu, H.; Chiba, S.; Sakata, T. Impaired response of UCP family to cold exposure in diabetic (db/db) mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2000**, *279*, R1305–R1309. DOI: 10.1152/ajpregu.2000.279.4.R1305.

Zhao, Y.; Dong, X.; Guo, C.-R.; Feng, L.; Liu, Y.; Jiang, W.-D.; Wu, P.; Luo, W.; Huang, X.-L.; Chen, D.-F. Dietary isoleucine affects muscle fatty acid and amino acid profiles through regulating lipid metabolism and autophagy in hybrid catfish Pelteobagrus vachelli♀× Leiocassis longirostris♂. *Anim. Nutr.* **2022**, DOI: 10.1016/j.aninu.2022.07.006.

Green, C.R.; Wallace, M.; Divakaruni, A.S.; Phillips, S.A.; Murphy, A.N.; Ciaraldi, T.P.; Metallo, C.M. Branched-chain amino acid catabolism fuels adipocyte differentiation and lipogenesis. *Nat. Chem. Biol.* **2016**, *12*, 15–21. DOI: 10.1038/nchembio.1961.

Luo, Y.; Zhang, X.; Zhu, Z.; Jiao, N.; Qiu, K.; Yin, J. Surplus dietary isoleucine intake enhanced monounsaturated fatty acid synthesis and fat accumulation in skeletal muscle of finishing pigs. *J. Anim. Sci. Biotechnol.* **2018**, *9*, 1–11. DOI: 10.1186/s40104-018-0306-5.

Doi, M.; Yamaoka, I.; Fukunaga, T.; Nakayama, M. Isoleucine, a potent plasma glucose-lowering amino acid, stimulates glucose uptake in C2C12 myotubes. *Biochem. Biophys. Res. Commun.* **2003**, *312*, 1111–1117. DOI: 10.1016/j.bbrc.2003.11.039.

Doi, M.; Yamaoka, I.; Nakayama, M.; Sugahara, K.; Yoshizawa, F. Hypoglycemic effect of isoleucine involves increased muscle glucose uptake and whole body glucose oxidation and decreased hepatic gluconeogenesis. *Am. J. Physiol. - Endocrinol. Metab.* **2007**, *292,* E1683–E1693. DOI: 10.1152/ajpendo.00609.2006.

Nishitani, S.; Takehana, K.; Fujitani, S.; Sonaka, I. Branched-chain amino acids improve glucose metabolism in rats with liver cirrhosis. *Am. J. Physiol. - Gastrointest. Liver Physiol.* **2005**, *288,* G1292–G1300. DOI: 10.1152/ajpgi.00510.2003.

Parr, T.; Kerr, B.; Baker, D. Isoleucine requirement of growing (25 to 45 kg) pigs. *J. Anim. Sci.* **2003**, *81,* 745–752. DOI: 10.2527/2003.813745x.

Wiltafsky, M.; Bartelt, J.; Relandeau, C.; Roth, F. Estimation of the optimum ratio of standardized ileal digestible isoleucine to lysine for eight-to twenty-five-kilogram pigs in diets containing spray-dried blood cells or corn gluten feed as a protein source. *J. Anim. Sci.* **2009**, *87*, 2554–2564. DOI: 10.2527/jas.2008-1320.

Oestemer, G.; Hanson, L.; Meade, R. Reevaluation of the isoleucine requirement of the young pig. *J. Anim. Sci.* **1973**, *36,* 679–683. DOI: 10.2527/jas1973.364679x.

Coude, F.; Sweetman, L.; Nyhan, W. Inhibition by propionyl-coenzyme A of N-acetylglutamate synthetase in rat liver mitochondria. A possible explanation for hyperammonemia in propionic and methylmalonic acidemia. *J. Clin. Investig.* **1979**, *64,* 1544–1551. DOI: 10.1172/JCI109614.

Stewart, P.M.; Walser, M. Failure of the normal ureagenic response to amino acids in organic acid-loaded rats: Proposed mechanism for the hyperammonemia of propionic and methylmalonic acidemia. *J. Clin. Investig.* **1980**, *66,* 484–492. DOI: 10.1172/JCI109879.

Scholl-Bürgi, S.; Sass, J.O.; Zschocke, J.; Karall, D. Amino acid metabolism in patients with propionic acidaemia. *J. Inherited Metab. Dis.* **2012**, *35*, 65–70. DOI: 10.1007/s10545-010-9245-9.

Nørgaard, J.; Fernández, J. Isoleucine and valine supplementation of crude protein-reduced diets for pigs aged 5–8 weeks. *Anim. Feed Sci. Technol.* **2009**, *154*, 248–253. DOI: 10.1016/j.anifeedsci.2009.08.010.

Zhang, X.; Liu, X.; Jia, H.; He, P.; Mao, X.; Qiao, S.; Zeng, X. Valine supplementation in a reduced protein diet regulates growth performance partially through modulation of plasma amino acids profile, metabolic responses, endocrine, and neural factors in piglets. *J. Agric. Food Chem.* **2018**, *66*, 3161–3168. DOI: 10.1021/acs.jafc.8b01113.

Ma, W.; Mao, P.; Fan, W.; Zhu, Y.; Guo, L. Valine and isoleucine supplementation improve performance and serum biochemical concentrations in growing gilts fed low-protein diets. *Can J Anim Sci* **2019**, *99*, 921-928.

Roux, M.; Donsbough, A.; Waguespack, A.; Powell, S.; Bidner, T.; Payne, R.; Southern, L. Maximizing the use of supplemental amino acids in corn-soybean meal diets for 20-to 45-kilogram pigs. *J. Anim. Sci.* **2011**, *89*, 2415–2424. DOI: 10.2527/jas.2010-3756.

Escobar, J.; Frank, J.W.; Suryawan, A.; Nguyen, H.V.; Kimball, S.R.; Jefferson, L.S.; Davis, T.A. Regulation of cardiac and skeletal muscle protein synthesis by individual branched-chain amino acids in neonatal pigs. *Am. J. Physiol. - Endocrinol. Metab.* **2006**, *290*, E612–E621. DOI: 10.1152/ajpendo.00402.2005.

Tischler, M.E.; Desautels, M.; Goldberg, A.L. Does leucine, leucyl-tRNA, or some metabolite of leucine regulate protein synthesis and degradation in skeletal and cardiac muscle? *J. Biol. Chem.* **1982**, *257*, 1613–1621. DOI: 10.1016/S0021-9258(19)68081-6

Chen, X.-h.; Liu, S.-r.; Peng, B.; Li, D.; Cheng, Z.-x.; Zhu, J.-x.; Zhang, S.; Peng, Y.-m.; Li, H.; Zhang, T.-t. Exogenous L-valine promotes phagocytosis to kill multidrug-resistant bacterial pathogens. *Front. Immunol.* **2017**, *8*, 207. DOI: 10.3389/fimmu.2017.00207.

Che, L.; Xu, M.; Gao, K.; Zhu, C.; Wang, L.; Yang, X.; Wen, X.; Xiao, H.; Jiang, Z.; Wu, D.J.B.O.R. Valine increases milk fat synthesis in mammary gland of gilts through stimulating AKT/MTOR/SREBP1 pathway. *Biol. Reprod.* **2019**, *101*, 126–137. DOI: 10.1093/biolre/ioz065.

Claessens, M.; Saris, W.H.; Bouwman, F.G.; Evelo, C.T.; Hul, G.B.; Blaak, E.E.; Mariman, E.C. Differential valine metabolism in adipose tissue of low and high fat-oxidizing obese subjects. *Proteomics Clin. Appl.* **2007**, *1*, 1306–1315. DOI: 10.1002/prca.200700049.

Du, Y.; Meng, Q.; Zhang, Q.; Guo, F. Isoleucine or valine deprivation stimulates fat loss via increasing energy expenditure and regulating lipid metabolism in WAT. *Amino Acids* **2012**, *43*, 725–734. DOI: 10.1007/s00726-011-1123-8.

Asghari, G.; Farhadnejad, H.; Teymoori, F.; Mirmiran, P.; Tohidi, M.; Azizi, F. High dietary intake of branched-chain amino acids is associated with an increased risk of insulin resistance in adults. *J. Diabetes* **2018**, *10*, 357–364. DOI: 10.1111/1753-0407.12639.

Craig, A.; Henry, W.; Magowan, E. Effect of phase feeding and valine-to-lysine ratio during lactation on sow and piglet performance. *J. Anim. Sci.* **2016**, *94*, 3835–3843. DOI: 10.2527/jas.2016-0648

Ma, Q.; Hu, L.; Zhu, J.; Chen, J.; Wang, Z.; Yue, Z.; Qiu, M.; Shan, A. Valine supplementation does not reduce lipid accumulation and improve insulin sensitivity in mice fed high-fat diet. *ACS omega* **2020**, *5*, 30937–30945. DOI: 10.1021/acsomega.0c03707.

Fukuchi, S.; Hamaguchi, K.; Seike, M.; Himeno, K.; Sakata, T.; Yoshimatsu, H. Role of fatty acid composition in the development of metabolic disorders in sucrose-induced obese rats. *Exp. Biol. Med.* **2004**, *229*, 486–493. DOI: 10.1177/153537020422900

Buettner, R.; Parhofer, K.; Woenckhaus, M.; Wrede, C.; Kunz-Schughart, L.A.; Scholmerich, J.; Bollheimer, L.J.J.o.m.e. Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. *J. Mol. Endocrinol.* **2006**, *36*, 485-501. DOI: 10.1677/jme.1.01909

Wan, J.; Jiang, L.; Lü, Q.; Ke, L.; Li, X.; Tong, N. Activation of PPARδ up-regulates fatty acid oxidation and energy uncoupling genes of mitochondria and reduces palmitate-induced apoptosis in pancreatic β-cells. *Biochem. Biophys. Res. Commun.* **2010**, *391*, 1567–1572. DOI: 10.1016/j.bbrc.2009.12.127.

Coskun, T.; Bina, H.A.; Schneider, M.A.; Dunbar, J.D.; Hu, C.C.; Chen, Y.; Moller, D.E.; Kharitonenkov, A. Fibroblast growth factor 21 corrects obesity in mice. *Endocrinology* **2008**, *149*, 6018–6027. DOI: 10.1210/en.2008-0816.

Lin, Z.; Tian, H.; Lam, K.S.; Lin, S.; Hoo, R.C.; Konishi, M.; Itoh, N.; Wang, Y.; Bornstein, S.R.; Xu, A. Adiponectin mediates the metabolic effects of FGF21 on glucose homeostasis and insulin sensitivity in mice. *Cell Metab.* **2013**, *17*, 779–789. DOI: 10.1016/j.cmet.2013.04.005.

Badman, M.K.; Pissios, P.; Kennedy, A.R.; Koukos, G.; Flier, J.S.; Maratos-Flier, E. Hepatic fibroblast growth factor 21 is regulated by PPARα and is a key mediator of hepatic lipid metabolism in ketotic states. *Cell Metab.* **2007**, *5*, 426–437. DOI: 10.1016/j.cmet.2007.05.002.

Inagaki, T.; Dutchak, P.; Zhao, G.; Ding, X.; Gautron, L.; Parameswara, V.; Li, Y.; Goetz, R.; Mohammadi, M.; Esser, V. Endocrine regulation of the fasting response by PPARα-mediated induction of fibroblast growth factor 21. *Cell Metab.* **2007**, *5,* 415–425. DOI: 10.1016/j.cmet.2007.05.003.

Bishop, C.A.; Schulze, M.B.; Klaus, S.; Weitkunat, K. The branched-chain amino acids valine and leucine have differential effects on hepatic lipid metabolism. *The FASEB Journal* **2020**, *34,* 9727–9739. DOI: 10.1096/fj.202000195R.

Imamura, F.; Fretts, A.; Marklund, M.; Ardisson Korat, A.V.; Yang, W.-S.; Lankinen, M.; Qureshi, W.; Helmer, C.; Chen, T.-A.; Wong, K. Fatty acid biomarkers of dairy fat consumption and incidence of type 2 diabetes: a pooled analysis of prospective cohort studies. *PLoS Med.* **2018**, *15,* e1002670. DOI: 10.1371/journal.pmed.1002670.

Jang, C.; Oh, S.F.; Wada, S.; Rowe, G.C.; Liu, L.; Chan, M.C.; Rhee, J.; Hoshino, A.; Kim, B.; Ibrahim, A. A branched-chain amino acid metabolite drives vascular fatty acid transport and causes insulin resistance. *Nat. Med.* **2016**, *22,* 421–426. DOI: 10.1038/nm.4057.

Arrieta-Cruz, I.; Su, Y.; Gutiérrez-Juárez, R. Suppression of endogenous glucose production by isoleucine and valine and impact of diet composition. *Nutrients* **2016**, *8,* 79. DOI: 10.3390/nu8020079.

Liao, X.; Liu, B.; Qu, H.; Zhang, L.; Lu, Y.; Xu, Y.; Lyu, Z.; Zheng, H. A high level of circulating valine is a biomarker for type 2 diabetes and associated with the hypoglycemic effect of sitagliptin. *Mediators Inflamm.* **2019**, *2019,* DOI: 10.1155/2019/8247019.

Elovaris, R.A.; Bitarafan, V.; Agah, S.; Ullrich, S.S.; Lange, K.; Horowitz, M.; Feinle-Bisset, C. Comparative Effects of the Branched-Chain Amino Acids, Leucine, Isoleucine and Valine,

on Gastric Emptying, Plasma Glucose, C-Peptide and Glucagon in Healthy Men. *Nutrients* **2021**, *13*, 1613. DOI: 10.3390/nu13051613.

Kimball, S.R.; Jefferson, L.S. Regulation of protein synthesis by branched-chain amino acids. *Curr. Opin. Clin. Nutr. Metab. Care* **2001**, *4*, 39–43.

Saheki, T.; Sato, Y.; Takada, S. Regulation of Urea Synthesis in Rat Liver Inhibition of Urea Synthesis by L-Norvaline. *J. Biochem.* **1979**, *86*, 745–750.

Bode, A.M.; Foster, J.D.; Nordlie, R.C. Glycogenesis from glucose and ureagenesis in isolated perfused rat livers. Influence of ammonium ion, norvaline, and ethoxyzolamide. *J. Biol. Chem.* **1994**, *269*, 7879–7886. DOI: 10.1016/S0021-9258(17)37133-8.

Polis, B.; Samson, A.O. A new perspective on Alzheimer's disease as a brain expression of a complex metabolic disorder. *Alzheimer's Disease [Internet]* **2019**, DOI: 10.15586/alzheimersdisease.2019.ch1.

Katunuma, N.; Okada, M.; Nishii, Y. Regulation of the urea cycle and TCA cycle by ammonia. *Adv. Enzyme Regul.* **1966**, *4*, 317–335. DOI: 10.1016/0065-2571(66)90025-2.

Liu, Y.; Wang, X.; Hou, Y.; Yin, Y.; Qiu, Y.; Wu, G.; Hu, C.-A.A. Roles of amino acids in preventing and treating intestinal diseases: recent studies with pig models. *Amino Acids* **2017**, *49*, 1277–1291. DOI: 10.1007/s00726-017-2450-1.

Verma, N.; Rettenmeier, A.W.; Schmitz-Spanke, S. Recent advances in the use of Sus scrofa (pig) as a model system for proteomic studies. *Proteomics* **2011**, *11*, 776–793. DOI: 10.1002/pmic.201000320.

Clouard, C.; Meunier-Salaün, M.-C.; Val-Laillet, D. Food preferences and aversions in human health and nutrition: how can pigs help the biomedical research? *Animal* **2012**, *6*, 118–136. DOI: 10.1017/S1751731111001315.

Spurlock, M.E.; Gabler, N.K. The development of porcine models of obesity and the metabolic syndrome. *J. Nutr.* **2008**, *138*, 397–402. DOI: 10.1093/jn/138.2.397.

Eberhard, M.; Hennig, U.; Kuhla, S.; Brunner, R.M.; Kleessen, B.; Metges, C.C. Effect of inulin supplementation on selected gastric, duodenal, and caecal microbiota and short chain fatty acid pattern in growing piglets. *Arch. Anim. Nutr.* **2007**, *61*, 235–246. DOI: 10.1080/17450390701431631.

Hou, N.; Du, X.; Wu, S. Advances in pig models of human diseases. *Exp. Anim.* **2022**, *5*, 141–152. DOI: 10.1002/ame2.12223.

Renner, S.; Braun-Reichhart, C.; Blutke, A.; Herbach, N.; Emrich, D.; Streckel, E.; Wünsch, A.; Kessler, B.; Kurome, M.; Bähr, A. Permanent neonatal diabetes in INS C94Y transgenic pigs. *Diabetes* **2013**, *62*, 1505–1511. DOI: 10.2337/db12-1065.

Cho, B.; Kim, S.J.; Lee, E.-J.; Ahn, S.M.; Lee, J.S.; Ji, D.-Y.; Lee, K.; Kang, J.-T. Generation of insulin-deficient piglets by disrupting INS gene using CRISPR/Cas9 system. *Transgenic Res.* **2018**, *27*, 289–300. DOI: 10.1007/s11248-018-0074-1.

Benevenga, N. Amino acid metabolism in swine: applicability to normal and altered amino acid metabolism in humans. **1986**, 1017–1030.

Oli, M.; Petschow, B.; Buddington, R. Evaluation of fructooligosaccharide supplementation of oral electrolyte solutions for treatment of diarrhea (Recovery of the intestinal bacteria). *Dig. Dis. Sci.* **1998**, *43*, 138–147. DOI: 10.1023/A:1018892524790.

Andersen, A.; Mølbak, L.; Thymann, T.; Michaelsen, K.; Lauritzen, L. Dietary long-chain n-3 PUFA, gut microbiota and fat mass in early postnatal piglet development—exploring a potential interplay. *Prostaglandins Leukot. Essent. Fat. Acids* **2011**, *85*, 345–351. DOI: 10.1016/j.plefa.2011.08.004.

Ananth, C.V.; Vintzileos, A.M. Distinguishing pathological from constitutional small for gestational age births in population-based studies. Early Hum. Dev. 2009, 85, 653–658, doi:10.1016/j.earlhumdev.2009.09.004.

Bernstein, I.; Gabbe, S.G. Intrauterine growth restriction. In Obstetrics: Normal and Problem Pregnancies, 3rd ed.; Gabbe, S.G., Niebyl, J.R., Simpson, J.L., Annas, G.J., et al., Eds.; Churchill Livingstone: New York, NY, USA, 1996; Volume 3, pp. 863–886.

Creasy, R.K.; Resnik, R. Intrauterine growth restriction. In Maternal-Fetal Medicine: Principles and Practice, 3rd ed.; Creasy, R.K., Resnik, R., Eds.; Saunders: Philadelphia, PA, USA, 1994; pp. 558–574.

Martin, J.A.; Hamilton, B.E.; Osterman, M.J.K.; Driscoll, A.K. Births: Final Data for 2019. National Vital Statistics Reports; National Center for Health Statistics: Hyattsville, MD, USA, 2021; Volume 70, doi:10.15620/cdc:100472.

Ewing, A.C.; Ellington, S.R.; Shapiro-Mendoza, C.K.; Barfield, W.D.; Kourtis, A.P. Full-term small-for-gestational-age newborns in the US: Characteristics, trends, and morbidity. Matern. Child Health J. 2017, 21, 786–796, doi:10.1007/s10995-016-2165-z.

Fontaine, M.A.; Diane, A.; Singh, V.P.; Mangat, R.; Krysa, J.A.; Nelson, R.; Willing, B.P.; Proctor, S.D. Low birth weight causes insulin resistance and aberrant intestinal lipid

metabolism independent of microbiota abundance in Landrace–Large White pigs. FASEB J. 2019, 33, 9250–9262, doi:10.1096/fj.201801302RR.

Giapros, V.; Vavva, E.; Siomou, E.; Kolios, G.; Tsabouri, S.; Cholevas, V.; Bairaktari, E.; Tzoufi, M.; Challa, A. Low-birth-weight, but not catch-up growth, correlates with insulin resistance and resistin level in SGA infants at 12 months. J. Matern. Fetal. Neonatal. Med. 2017, 30, 1771–1776, doi:10.1080/14767058.2016.1224838.

Li, N.; Wang, W.; Wu, G.; Wang, J. Nutritional support for low birth weight infants: Insights from animal studies. Br. J. Nutr. 2017, 117, 1390–1402, doi:10.1017/S000711451700126X.

Wang, X.; Cui, Y.; Tong, X.; Ye, H.; Li, S. Glucose and lipid metabolism in small-for-gestational-age infants at 72 hours of age. J. Clin. Endocrinol. Metab. 2007, 92, 681–684, doi:10.1210/jc.2006-1281.

Xu, Y.; Chen, S.; Yang, H.; Gong, F.; Wang, L.; Jiang, Y.; Yan, C.; Zhu, H.; Pan, H. Decreased insulin sensitivity and abnormal glucose metabolism start in preadolescence in low-birth-weight children—Meta-analysis and systematic review. Prim. Care Diabetes. 2019, 13, 391–398, doi:10.1016/j.pcd.2019.03.012.

Lane, R.H.; Flozak, A.S.; Ogata, E.S.; Bell, G.I.; Simmons, R.A. Altered hepatic gene expression of enzymes involved in energy metabolism in the growth-retarded fetal rat. Pediatr. Res. 1996, 39, 390–394, doi:10.1203/00006450-199603000-00003.

Peterside, I.E.; Selak, M.A.; Simmons, R.A. Impaired oxidative phosphorylation in hepatic mitochondria in growth-retarded rats. Am. J. Physiol. Endocrinol. Metab. 2003, 285, 1258–1266, doi:10.1152/ajpendo.00437.2002.

Gomo, Z.A.R.; Nyatanga, K.; Chifamba, J.; Chinyanga, H.M.; Taderera, T.; Gwaunza, L.T.; Mushayamano, T.; Mahachi, C. Glucose tolerance study in low and normal birth weight young adults. Cent. Afr. J. Med. 2013, 59, 38–42.

Ying, Z.; Ge, X.; Zhang, H.; Su, W.; Li, Y.; Zhou, L.; Zhang, L.; Wang, T. Effects of dietary methionine restriction on postnatal growth, insulin sensitivity, and glucose metabolism in intrauterine growth retardation pigs at 49 and 105 d of age. J. Anim. Sci. 2019, 97, 610–619, doi:10.1093/jas/sky457.

Ying, Z.; Zhang, H.; Su, W.; Zhou, L.; Wang, F.; Li, Y.; Zhang, L.; Wang, T. Dietary methionine restriction alleviates hyperglycemia in pigs with intrauterine growth restriction by enhancing hepatic protein kinase B signaling and glycogen synthesis. J. Nutr. 2017, 147, 1892–1899, doi:10.3945/jn.117.253427.

Jones, J.N.; Gercel-Taylor, C.; Taylor, D.D. Altered cord serum lipid levels associated with small for gestational age infants. Obstet. Gynecol. 1999, 93, 527–531, doi:10.1016/S0029-7844(98)00489-X.

Liu, C.; Lin, G.; Wang, X.; Wang, T.; Wu, G.; Li, D.; Wang, J. Intrauterine growth restriction alters the hepatic proteome in fetal pigs. J. Nutr. Biochem. 2013, 24, 954–959, doi:10.1016/j.jnutbio.2012.06.016.

Cooper, J.E. The use of the pig as an animal model to study problems associated with low birthweight. Lab. Anim. 1975, 9, 329–336, doi:10.1258/002367775780957188.

Ferenc, K.; Pietrzak, P.; Godlewski, M.M.; Piwowarski, J.; Kilianczyk, R.; Guilloteau, P.; Zabielski, R. Intrauterine growth retarded piglet as a model for humans–studies on the perinatal

development of the gut structure and function. Reprod. Biol. 2014, 14, 51–60, doi:10.1016/j.repbio.2014.01.005.

Eiby, Y.A.; Wright, L.L.; Kalanjati, V.P.; Miller, S.M.; Bjorkman, S.T; Keates, H.L.; Lumbers, E.R.; Colditz, P.B.; Lingwood, B.E. A pig model of the preterm neonate: Anthropometric and physiological characteristics. PLoS ONE 2013, 8, e68763, doi:10.1371/journal.pone.0068763.

Odle, J.; Lin, X.; Jacobi, S.K.; Kim, S.W; Stahl, C.H. The suckling piglet as an agrimedical model for the study of pediatric nutrition and metabolism. Annu. Rev. Anim. Biosci. 2014, 2, 419–444, doi:10.1146/annurev-animal-022513-114158.

Ardiansyah, S.; Shirakawa, H.; Inagawa, Y.; Koseki, T.; Komai, M. Regulation of blood pressure and glucose metabolism induced by L-tryptophan in stroke-prone spontaneously hypertensive rats. Nutr. Metab. 2011, 8, 1–7, doi:10.1186/1743-7075-8-45.

Ruan, Z.; Yang, Y.; Wen, Y.; Zhou, Y.; Fu, X.; Ding, S.; Liu, G.; Yao, K.; Wu, X.; Deng, Z.; Wu, G.; Yin, Y. Metabolomic analysis of amino acid and fat metabolism in rats with l-tryptophan supplementation. Amino Acids 2014, 46, 2681–2691, doi:10.1007/s00726-014-1823-y.

Akiba, Y.; Takahashi, K.; Horiguchi, M.; Ohtani, H.; Saitoh, S.; Ohkawara, H. L-tryptophan alleviates fatty liver and modifies hepatic microsomal mixed function oxidase in laying hens. Comp. Biochem. Physiol. A Physiol. 1992, 102, 769–774, doi:10.1016/0300-9629(92)90738-c.

Rogers, S.R.; Pesti, G.M. The influence of dietary tryptophan on broiler chick growth and lipid metabolism as mediated by dietary protein levels. Poult. Sci. 1990, 69, 746–756, doi:10.3382/ps.0690746.

Inubushi, T.; Kamemura, N.; Oda, M.; Sakurai, J.; Nakaya, Y.; Harada, N.; Suenaga, M.; Matsunaga, Y.; Ishidoh, K.; Katunuma, N. L-tryptophan suppresses rise in blood glucose and preserves insulin secretion in type-2 diabetes mellitus rats. J. Nutr. Sci. Vitaminol. 2012, 58, 415–422, doi:10.3177/jnsv.58.415.

Willemen, S.A.; Che, L.; Dewilde, S.; Van Hauwaert, M.L.; De Vos, M.; Huygelen, V.; Fransen, E.; Tambuyzer, B.R.; Casteleyn, C.; Van Cruchten, S.; Van Ginneken, C. Enteric and serological distribution of serotonin and its precursor tryptophan in perinatal low and normal weight piglets. Animals 2014, 8, 792–799, doi:10.1017/S1751731114000317.

Cui, R.J.; Roberts, B.L.; Zhao, H.; Zhu, M.; Appleyard, S.M. Serotonin activates catecholamine neurons in the solitary tract nucleus by increasing spontaneous glutamate inputs. J. Neurosci. 2012, 32, 16530–16538, doi:10.1523/JNEUROSCI.1372-12.

Fetissov, S.O.; Meguid, M.M.; Chen, C.; Miyata, G. Synchronized release of dopamine and serotonin in the medial and lateral hypothalamus of rats. Neuroscience 2000, 101, 657–663, doi:10.1523/JNEUROSCI.1372-12.2012.

Lam, D.D.; Garfield, A.S.; Marston, O.J.; Shaw, J.; Heisler, L.K. Brain serotonin system in the coordination of food intake and body weight. Pharmacol. Biochem. Behav. 2010, 97, 84–91, doi:10.1016/j.pbb.2010.09.003.

Dépôt, M.; Caillé, G.; Mukherjee, J.; Katzman, M.A.; Cadieux, A.; Bradwejn, J. Acute and chronic role of 5-HT3 neuronal system on behavioral and neuroendocrine changes induced by intravenous cholecystokinin tetrapeptide administration in humans. Neuropsychopharmacology 1999, 20, 177–187, doi:10.1016/S0893-133X(98)00074-8.

McGlone, J. Guide for the Care and Use of Agricultural Animals in Research and Teaching, 3rd ed. Available online: https://www.aaalac.org/about/ag_guide_3rd_ed.pdf (accessed on 4 March 2019).

Rafiee-Tari, N.; Fan, M.Z.; Archbold, T.; Arranz, E.; Corredig, M. Effect of milk protein composition and amount of β-casein on growth performance, gut hormones, and inflammatory cytokines in an in vivo piglet model. J. Dairy Sci. 2019, 102, 8604–8613, doi:10.3168/jds.2018-15786.

Wang, Y.; Zhang, L.; Zhou, G.; Liao, Z.; Ahmad, H.; Liu, W.; Wang, T. Dietary L-arginine supplementation improves the intestinal development through increasing mucosal Akt and mammalian target of rapamycin signals in intra-uterine growth retarded piglets. Br. J. Nutr. 2012, 108, 1371–1381, doi:10.1017/S0007114511006763.

Zheng, P.; Song, Y.; Tian,Y.; Zhang, H.; Yu, B.; He, J.; Mao, X.; Yu, J.; Luo, Y.; Luo, J.; Huang, Z.; Tian, G.; Chen, H.; Chen, D. Dietary arginine supplementation affects intestinal function by enhancing antioxidant capacity of a nitric oxide–independent pathway in low-birth-weight piglets. J. Nutr. 2018, 148, 1751–1759, doi:10.1093/jn/nxy198.

DeRouchey, J.M.; Goodband, R.D.; Tokach, M.D.; Nelssen, J.L.; Dritz, S.S. Nursery Swine Nutrient Recommendations and Feeding Management. In National Swine Nutrition Guide; Meisinger, D.J. Ed.; U.S. Pork Center of Excellence: Ames, IA, USA, 2010; pp. 65–79.

Ebert, A.R.; Berman, A,S.; Harrell, R.J.; Kessler, A.M.; Cornelius, S.G.; Odle, J. Vegetable proteins enhance the growth of milk-fed piglets, despite lower apparent ileal digestibility. J. Nutr. 2005, 135, 2137–2143, doi:10.1093/jn/135.9.2137.

Habibi, M.; Shili, C.; Sutton, J.; Goodarzi, P.; Maylem, E.R;. Spicer, L.; Pezeshki, A. Branched-chain amino acids partially recover the reduced growth of pigs fed with protein-restricted diets through both central and peripheral factors. Anim. Nutr. 2021, doi:10.1016/j.aninu.2021.02.002.

Kim, S.W.; McPherson, R.L.; Wu, G. Dietary arginine supplementation enhances the growth of milk-fed young pigs. J. Nutr. 2004, 134, 625–630, doi:10.1093/jn/134.3.625.

Madsen, J.G.; Mueller, S.; Kreuzer, M.; Bigler, M.B.; Silacci, P.; Bee, G. Milk replacers supplemented with either L-arginine or L-carnitine potentially improve muscle maturation of early reared low birth weight piglets from hyperprolific sows. Animals 2018, 12, 43–53, doi:10.1017/S175173111700132X.

Jensen, G.M.; Grøndahl, M.L.; Nielsen, C.G.; Skadhauge, E.; Olsen, J.E.; Hansen, M.B. Effect of ondansetron on Salmonella typhimurium-induced net fluid accumulation in the pig jejunum in vivo. Comp. Biochem. Physiol. A Physiol. 1997, 118, 297–299, doi:10.1016/s0300-9629(96)00308-8.

Spring, S.; Premathilake, H.; Bradway, C.; Shili, C.; DeSilva, U.; Carter, S.; Pezeshki, A. Effect of very low-protein diets supplemented with branched-chain amino acids on energy balance, plasma metabolomics and fecal microbiome of pigs. Sci. Rep. 2020, 10,1–16, doi:10.1038/s41598-020-72816-8.

Spring, S.; Premathilake, H.; DeSilva, U.; Shili, C.; Carter, S.; Pezeshki, A. Low protein-high carbohydrate diets alter energy balance, gut microbiota composition and blood metabolomics profile in young pigs. Sci. Rep. 2020, 10, 1–15, doi:10.1038/s41598-020-60150-y.

Shili, C.N.; Broomhead, J.N.; Spring, S.C.; Lanahan, M.B.; Pezeshki, A. A novel corn-expressed phytase improves daily weight gain, protein efficiency ratio and nutrients digestibility and alters fecal microbiota in pigs fed with very low protein diets. Animals 2020, 10, 1926, doi:10.3390/ani10101926.

AOAC. Official Method 982.30 E (a, b, c), chp, 45.3. 05 2006. Amino Acid profile without Tryptophan AAP-T; Association of Official Analytical Chemists: Gaithersburg, USA, 2006.

Bakken, G.S. A heat transfer analysis of animals: Unifying concepts and the application of metabolism chamber data to field ecology. J. Theor. Biol. 1976, 60, 337–384, doi:10.1016/0022-5193(76)90063-1.

Ferreira, V.M.; Francisco, N.S.; Belloni, M.; Aguirre, G.M.; Caldara, F.R.; Nääs, I.A.; Garcia, R.G.; Almeida Paz, I.C.; Polycarpo, G.V. Infrared thermography applied to the evaluation of metabolic heat loss of chicks fed with different energy densities. Braz. J. Poult. Sci. 2011, 13, 113–118, doi:10.1590/S1516-635X2011000200005.

Digalakis, M.; Papamichail, M.; Glava, C.; Grammatoglou, X.; Sergentanis, T.N.; Papalois, A.; Bramis, J. Interposition of a reversed jejunal segment enhances intestinal adaptation in short bowel syndrome: An experimental study on pigs. J. Surg. Res. 2011, 171, 551–557, doi:10.1016/j.jss.2010.06.035.

Pezeshki, A.; Muench, G.P.; Chelikani, P.K. Short communication: Expression of peptide YY, proglucagon, neuropeptide Y receptor Y2, and glucagon-like peptide-1 receptor in bovine peripheral tissues. J. Dairy Sci. 2012, 95, 5089–5094, doi:10.3168/jds.2011-5311.

Shili, C.N.; Habibi, M.; Sutton, J.; Barnes, J.; Burch-Konda, J.; Pezeshki, A. Effect of a phytogenic water additive on growth performance, blood metabolites and gene expression of

amino acid transporters in nursery pigs fed with low-protein/high-carbohydrate diets. Animals 2021, 11, 555, doi:10.3390/ani11020555.

Chen, G.; Zhang, J.; Zhang, Y.; Liao, P.; Li, T.; Chen, L.; Yin, Y.; Wang, J.; Wu, G. Oral MSG administration alters hepatic expression of genes for lipid and nitrogen metabolism in suckling piglets. Amino Acids 2014, 46, 245–250, doi:10.1007/s00726-013-1615-9.

Duran-Montgé, P.; Theil, P.K.; Lauridsen, C.; Esteve-Garcia E. Dietary fat source affects metabolism of fatty acids in pigs as evaluated by altered expression of lipogenic genes in liver and adipose tissues. Animal 2009, 3, 535–542, doi:10.1017/S1751731108003686.

Li, S.; Wang, H.; Wang, X.; Wang, Y.; Feng, J. Betaine affects muscle lipid metabolism via regulating the fatty acid uptake and oxidation in finishing pig. J. Anim. Sci. Biotechnol. 2017, 8, 1–9, doi:10.1186/s40104-017-0200-6.

He, D.; Ma, J.; Long, K.; Wang, X.; Li, X.; Jiang, A.; Li, M. Differential expression of genes related to glucose metabolism in domesticated pigs and wild boar. Biosci. Biotechnol. Biochem. 2017, 81, 1478–1483, doi:10.1080/09168451.2017.1322893.

Kang, P.; Liu, Y.; Zhu, H.; Zhang, J.; Shi, H.; Li, S.; Pi, D.; Leng, W.; Wang, X.; Wu, H.; Hou, Y. The effect of dietary asparagine supplementation on energy metabolism in liver of weaning pigs when challenged with lipopolysaccharide. Asian Australas J. Anim. Sci. 2018, 31, 548–555, doi:10.5713/ajas.17.0426.

Xie, C.; Wang, Q.; Wang, J.; Tan, B.; Fan, Z.; Deng, Z.Y.; Wu, X.; Yin, Y. Developmental changes in hepatic glucose metabolism in a newborn piglet model: A comparative analysis for suckling period and early weaning period. Biochem. Biophys. Res. Commun. 2016, 470, 824–830, doi:10.1016/j.bbrc.2016.01.114.

Yin, J.; Liu, M.; Ren, W.; Duan, J.; Yang, G.; Zhao, Y.; Fang, R.; Chen, L.; Li, T.; Yin, Y. Effects of dietary supplementation with glutamate and aspartate on diquat-induced oxidative stress in piglets. PLoS ONE 2015, 10, e0122893, doi:10.1371/journal.pone.0122893.

Zuo, F.; Gu, Q.; Li, S.; Wei, H.; Peng, J. Effects of Different Methionine Sources on Methionine Metabolism in the IPEC-J2 Cells. Biomed. Res. Int. 2019, 1–11, doi:10.1155/2019/5464906.

Pezeshki; A.; Chelikani, P.K. Effects of Roux-en-Y gastric bypass and ileal transposition surgeries on glucose and lipid metabolism in skeletal muscle and liver. Surg. Obes. Relat. Dis. 2014, 10, 217–228, doi:10.1016/j.soard.2013.09.017.

Pezeshki, A.; Zapata, R.C.; Singh, A.; Yee, N.J.; Chelikani, P.K. Low protein diets produce divergent effects on energy balance. Sci. Rep. 2016, 6, 1–13, doi:10.1038/srep25145.

Pezeshki, A.; Fahim, A.; Chelikani, P.K. Dietary whey and casein differentially affect energy balance, gut hormones, glucose metabolism, and taste preference in diet-induced obese rats. J. Nutr. 2015, 145, 2236–2244, doi:10.3945/jn.115.213843.

Smith, S.A.; Pogson, C.L. Tryptophan and the control of plasma glucose concentrations in the rat. Biochem. J. 1977, 168, 495–506, doi:10.1042/bj1680495.

Ullrich, S.S.; Fitzgerald, P.C.E.; Giesbertz, P.; Steinert, R.E.; Horowitz, M.; Feinle-Bisset, C. Effects of intragastric administration of tryptophan on the blood glucose response to a nutrient drink and energy intake, in lean and obese men. Nutrients 2018, 10, 463, doi:10.3390/nu10040463.

Ponter, A.A.; Sève, B.; Morgan, L.M. Intragastric tryptophan reduces glycemia after glucose, possibly via glucose-mediated insulinotropic polypeptide, in early-weaned piglets. J. Nutr. 1994, 124, 259–267, doi:10.1093/jn/124.2.259.

Hedo, J.A.; Villanueva, M.L.; Marco, J. Elevation of plasma glucose and glucagon after tryptophan ingestion in man. Metabolism 1977, 26, 1131–1134, doi:10.1016/0026-0495(77)90040-3.

Tsiolakis, D.; Marks, V. The differential effect of intragastric and intravenous tryptophan on plasma glucose, insulin, glucagon, GLI and GIP in the fasted rat. Horm. Metab. Res. 1984, 16, 226–229, doi:10.1055/s-2007-1014752.

Lin, H.V.; Efanov, A.M.; Fang, X.; Beavers, L.S.; Wang, X.; Wang, J.; Gonzalez Valcarcel, I.C.; Ma, T. GPR142 controls tryptophan-induced insulin and incretin hormone secretion to improve glucose metabolism. PLoS ONE 2016, 11, e0157298, doi:10.1371/journal.pone.0157298.

Hajishafiee, M.; Elovaris, R.A.; Jones, K.L.; Heilbronn, L.K.; Horowitz, M.; Poppitt, S.D.; Feinle-Bisset, C. Effects of intragastric administration of L-tryptophan on the glycaemic response to a nutrient drink in men with type 2 diabetes—impacts on gastric emptying, glucoregulatory hormones and glucose absorption. Nutr. Diabetes 2021, 11, 1–9, doi:10.1038/s41387-020-00146-9.

Ji, K.; Liang, H.; Chisomo-Kasiya, H.; Mokrani, A.; Ge, X.; Ren, M.; Liu, B. Effects of dietary tryptophan levels on growth performance, whole body composition and gene expression levels related to glycometabolism for juvenile blunt snout bream, Megalobrama amblycephala. Aquac. Nutr. 2018, 24, 1474–1483, doi:10.1111/anu.12684.

Iynedjian, P.B. Molecular physiology of mammalian glucokinase. Cell. Mol. Life Sci. 2009, 66, 27–42, doi:10.1007/s00018-008-8322-9.

Zelent, B.; Odili, S.; Buettger, C.; Shiota, C.; Grimsby, J.; Taub, R.; Magnuson, M.A.; Vanderkooi, J.M.; Matschinsky, F.M. Sugar binding to recombinant wild-type and mutant glucokinase monitored by kinetic measurement and tryptophan fluorescence. Biochem. J. 2008, 413, 269–280, doi:10.1042/BJ20071718.

Ray, P.D.; Foster, D.O.; Lardy, H.A. Paths of carbon in gluconeogenesis and lipogenesis: IV. Inhibition by L-tryptophan of hepatic gluconeogenesis at the level of phosphoenolpyruvate formation. J. Biol. Chem. 1966, 241, 3904–3908, doi:10.1016/S0021-9258(18)99792-9.

Foster, D.O.; Ray, P.D.; Lardy, H.A. A paradoxical in vivo effect of L-tryptophan on the phosphoenolpyruvate carboxykinase of rat liver. Biochemistry 1966, 5, 563–569, doi:10.1021/bi00866a023.

Rippe, D.F.; Berry, L.J. Effect of endotoxin on the activation of phosphoenolpyruvate carboxykinase by tryptophan. Infect. Immun. 1972, 6, 97–98, doi:10.1128/IAI.6.1.97-98.1972.

Quinn, P.G.; Yeagley, D. Insulin regulation of PEPCK gene expression: A model for rapid and reversible modulation. Curr. Drug Targets-Immune. Endocr. Metabol. Disord. 2005, 5, 423–437, doi:10.2174/156800805774912962.

Karim, S.; Adams, D.H.; Lalor, P.F. Hepatic expression and cellular distribution of the glucose transporter family. World J. Gastroenterol. 2012, 18, 6771–6781, doi:10.3748/wjg.v18.i46.6771.

Eleftheriadis, T.; Pissas, G.; Sounidaki, M.; Tsogka, K.; Antoniadis, N.; Antoniadi, G.; Liakopoulos, V.; Stefanidis, I. Indoleamine 2, 3-dioxygenase, by degrading L-tryptophan,

enhances carnitine palmitoyltransferase I activity and fatty acid oxidation, and exerts fatty acid-dependent effects in human alloreactive CD4+ T-cells. Int. J. Mol. Med. 2016, 38,1605–1613, doi:10.3892/ijmm.2016.2750.

Sun, H.; Huang, F.F.; Qu, S. Melatonin: A potential intervention for hepatic steatosis. Lipids Health Dis. 2015, 14, 1–6, doi:10.1186/s12944-015-0081-7.

Nishida, S.; Segawa, T.; Murai, I.; Nakagawa, S. Long-term melatonin administration reduces hyperinsulinemia and improves the altered fatty-acid compositions in type 2 diabetic rats via the restoration of Δ-5 desaturase activity. J. Pineal Res. 2002, 32, 26–33, doi:10.1034/j.1600-079x.2002.10797.x.

Gao, J.; Xu, K.; Liu, H.; Liu, G.; Bai, M.; Peng, C.; Li, T.; Yin, Y. Impact of the gut microbiota on intestinal immunity mediated by tryptophan metabolism. Front. Cell. Infect. Mi. 2018, 8, 13, doi:10.3389/fcimb.2018.00013.

Yu, Y.; Raka, F.; Adeli, K. The role of the gut microbiota in lipid and lipoprotein metabolism. J. Clin. Med. 2019, 8, 2227, doi:10.3390/jcm8122227.

Utzschneider, K.M.; Kratz, M.; Damman, C.J.; Hullarg, M. Mechanisms linking the gut microbiome and glucose metabolism. J. Clin. Endocrinol. Metab. 2016, 101, 1445–1454, doi:10.1210/jc.2015-4251.

Wenninger, J.; Meinitzer, A.; Holasek, S.; Schnedl, W.J.; Zelzer, S.; Mangge, H.; Herrmann, M.; Enko, D. Associations between tryptophan and iron metabolism observed in individuals with and without iron deficiency. Sci. Rep. 2019, 9, 1–9, doi:10.1038/s41598-019-51215-8.

Wessels, A.G.; Kluge, H.; Hirche, F.; Kiowski, A.; Bartelt, J.; Corrent, E.; Stangl, G.I. High leucine intake reduces the concentration of hypothalamic serotonin in piglets. J. Anim. Sci. 2016, 94, 26–29, doi:10.2527/jas.2015-9728.

van der Hoek, G.A.; Cooper, S.J. Ondansetron, a selective 5-HT3 receptor antagonist, reduces palatable food consumption in the nondeprived rat. Neuropharmacology 1994, 33, 805–811, doi:10.1016/0028-3908(94)90120-1.

Yabut, J.M.; Crane, J.D.; Green, A.E.; Keating, D.J.; Khan, W.I.; Steinberg, G.R. Emerging roles for serotonin in regulating metabolism: New implications for an ancient molecule. Endocr. Rev. 2019, 40, 1092–1107, doi:10.1210/er.2018-00283.

Namkung, J.; Kim, H.; Park, S. Peripheral serotonin: A new player in systemic energy homeostasis. Mol. Cell. 2015, 38, 1023–1028, doi:10.14348/molcells.2015.0258.

Schoenichen, C.; Bode, C.; Duerschmied, D. Role of platelet serotonin in innate immune cell recruitment. Front. Biosci. 2019, 24, 514–526, doi:10.2741/4732.

Christensen, R.D.; Henry, E.; Wiedmeier, S.E.; Stoddard, R.A.; Sola-Visner, M.C.; Lambert, D.K.; Kiehn, T.I.; Ainsworth, S. Thrombocytopenia among extremely low birth weight neonates: Data from a multihospital healthcare system. J. Perinatol. 2006, 26, 348–353, doi:10.1038/sj.jp.7211509.

Wang, Y.; Zhou, J.; Wang, G.; Cai, S.; Zeng, X.; Qiao, S. Advances in low-protein diets for swine. *J. Anim. Sci. Biotechnol.* **2018**, *9*, 1–14. DOI: 10.1186/s40104-018-0276-7

Lordelo, M.; Gaspar, A.; Le Bellego, L.; Freire, J. Isoleucine and valine supplementation of a low-protein corn-wheat-soybean meal-based diet for piglets: Growth performance and nitrogen balance. *J. Anim. Sci.* **2008**, *86*, 2936–2941. DOI: 10.2527/jas.2007-0222

Opapeju, F.; Rademacher, M.; Blank, G.; Nyachoti, C. Effect of low-protein amino acid-supplemented diets on the growth performance, gut morphology, organ weights and digesta characteristics of weaned pigs. *Animal* **2008**, *2*, 1457–1464. DOI: 10.1017/S175173110800270X

Le Bellego, L.; van Milgen, J.; Noblet, J. Effect of high temperature and low-protein diets on the performance of growing-finishing pigs. *J. Anim. Sci.* **2002**, *80*, 691–701. DOI: 10.2527/2002.803691x

Shriver, J.A.; Carter, S.D.; Sutton, A.L.; Richert, B.T.; Senne, B.W.; Pettey, L.A. Effects of adding fiber sources to reduced-crude protein, amino acid-supplemented diets on nitrogen excretion, growth performance, and carcass traits of finishing pigs. *J. Anim. Sci.* **2003**, *81*, 492–502. DOI: 10.2527/2003.812492x

Madrid, J.; Martínez, S.; López, C.; Orengo, J.; López, M.J.; Hernández, F. Effects of low protein diets on growth performance, carcass traits and ammonia emission of barrows and gilts. *Anim. Prod. Sci.* **2012**, *53*, 146–153. DOI: 10.1071/AN12067

Figueroa, J.; Lewis, A.; Miller, P.S.; Fischer, R.; Gómez, R.; Diedrichsen, R. Nitrogen metabolism and growth performance of gilts fed standard corn-soybean meal diets or low-crude protein, amino acid-supplemented diets. *J. Anim. Sci.* **2002**, *80*, 2911–2919. DOI: 10.2527/2002.80112911x

Deng, D.; Huang, R.L.; Li, T.J.; Wu, G.Y.; Xie, M.Y.; Tang, Z.R.; Kang, P.; Zhang, Y.M.; Fan, M.Z.; Kong, X.F.; Ruan, Z. Nitrogen balance in barrows fed low-protein diets supplemented with essential amino acids. *Livest. Sci.* **2007**, *109*, 220–223. DOI: 10.1016/j.livsci.2007.01.122

He, L.; Wu, L.; Xu, Z.; Li, T.; Yao, K.; Cui, Z.; Yin, Y.; Wu, G. Low-protein diets affect ileal amino acid digestibility and gene expression of digestive enzymes in growing and finishing pigs. *Amino acids* **2016**, *48*, 21–30. DOI: 10.1007/s00726-015-2059-1

Portejoie, S.; Dourmad, J.Y.; Martinez, J.; Lebreton, Y. Effect of lowering dietary crude protein on nitrogen excretion, manure composition and ammonia emission from fattening pigs. *Livest. Prod. Sci.* **2004**, *91*, 45–55. DOI: 10.1016/j.livprodsci.2004.06.013

Nyachoti, C.M.; Omogbenigun, F.O.; Rademacher, M.; Blank, G. Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. *J. Anim. Sci.* **2006**, *84*, 125–134. DOI: 10.2527/2006.841125x

Wu, L.; Liao, P.; He, Q.; Tan, B.; Guo, F.; Tang, M.; Li, T. Chronic feeding with protein-restricted diets affect ileal amino acid digestibility and the expression of nutrient-sensing, hormone secretion, gastrointestinal digestive enzyme, and nutrient transporter genes in young weaned pigs. *Oncotarget* **2018**, *5*, 1–15. Retrieved from https://www.oncotarget.com/article/24093/text/

Spring, S.; Premathilake, H.; DeSilva, U.; Shili, C.; Carter, S.; Pezeshki, A. Low protein-high carbohydrate diets alter energy balance, gut microbiota composition and blood metabolomics profile in young pigs. *Sci. Rep.* **2020**, *10*, 3318. DOI: 10.1038/s41598-020-60150-y

Luo, Z.; Li, C.; Cheng, Y.; Hang, S.; Zhu, W. Effects of low dietary protein on the metabolites and microbial communities in the caecal digesta of piglets. *Arch. Anim. Nutr.* **2015**, *69*, 212–226. DOI: 10.1080/1745039X.2015.1034521

NRC. *Nutrient Requirements of Swine: Eleventh Revised Edition*; The National Academies Press: Washington, DC, USA, 2012.

Spring, S.; Premathilake, H.; Bradway, C.; Shili, C.; DeSilva, U.; Carter, S.; Pezeshki, A. Effect of very low-protein diets supplemented with branched-chain amino acids on energy balance, plasma metabolomics and fecal microbiome of pigs. *Sci. Rep.* **2020**, *10*, 15859. DOI: 10.1038/s41598-020-72816-8

Habibi, M.; Shili, C.; Sutton, J.; Goodarzi, P.; Maylem, E.R.; Spicer, L.; Pezeshki, A. Branched-chain amino acids partially recover the reduced growth of pigs fed with protein-restricted diets through both central and peripheral factors. *Anim Nutr.* **2021**, *7*, 868–882. DOI: 10.1016/j.aninu.2021.02.002

Figueroa, J.; Lewis, A.; Miller, P.S.; Fischer, R.; Diedrichsen, R. Growth, carcass traits, and plasma amino acid concentrations of gilts fed low-protein diets supplemented with amino acids including histidine, isoleucine, and valine. *J. Anim. Sci.* **2003**, *81*, 1529–1537. DOI: 10.2527/2003.8161529x

Zhao, Y.; Tian, G.; Chen, D.; Zheng, P.; Yu, J.; He, J.; Mao, X.; Huang, Z.; Luo, Y.; Luo, J.; Yu, B. Effect of different dietary protein levels and amino acids supplementation patterns on growth performance, carcass characteristics and nitrogen excretion in growing-finishing pigs. *J. Anim. Sci. Biotechnol.* **2019**, *10*, 1–10. DOI: 10.1186/s40104-019-0381-2

Gloaguen, M.; Le Floc'H, N.; Brossard, L.; Barea, R.; Primot, Y.; Corrent, E.; van Milgen, J. Response of piglets to the valine content in diet in combination with the supply of other branched-chain amino acids. *Animal.* **2011**, *5*, 1734–1742. DOI: 10.1017/S1751731111000760

Gloaguen, M.; Le Floc'H, N.; Corrent, E.; Primot, Y.; van Milgen, J. Providing a diet deficient in valine but with excess leucine results in a rapid decrease in feed intake and modifies the postprandial plasma amino acid and α-keto acid concentrations in pigs. *J. Anim. Sci.* **2012**, *90*, 3135–3142. DOI: 10.2527/jas.2011-4956

Wiltafsky, M.K.; Pfaffl, M.W.; Roth, F.X. The effects of branched-chain amino acid interactions on growth performance, blood metabolites, enzyme kinetics and transcriptomics in weaned pigs. *Br. J. Nutr.* **2010**, *103*, 964–976. DOI: 10.1017/S0007114509992212

Edmonds, M.; Baker, D. Amino acid excesses for young pigs: Effects of excess methionine, tryptophan, threonine or leucine. *J. Anim. Sci.* **1987**, *64*, 1664–1671. DOI: 10.2527/jas1987.6461664x

Habibi, M.; Goodarzi, P.; Shili, C.N.; Sutton, J.; Wileman, C.M.; Kim, D.M.; Lin, D.; Pezeshki, A. A Mixture of Valine and Isoleucine Restores the Growth of Protein-Restricted Pigs Likely through Improved Gut Development, Hepatic IGF-1 Pathway, and Plasma Metabolomic Profile. *Int J Mol Sci.* **2022**, *23*, 3300. DOI: 10.3390/ijms23063300

Nørgaard, J.V.; Fernández, J.A. Isoleucine and valine supplementation of crude protein-reduced diets for pigs aged 5–8 weeks. *Anim. Feed Sci. Technol.* **2009**, *154*, 248–253. DOI: 10.1016/j.anifeedsci.2009.08.010

Zhang, X.; Liu, X.; Jia, H.; He, P.; Mao, X.; Qiao, S.; Zeng, X. Valine supplementation in a reduced protein diet regulates growth performance partially through modulation of plasma amino acids profile, metabolic responses, endocrine, and neural factors in piglets. *J. Agric. Food Chem.* **2018**, *66*, 3161–3168. DOI: 10.1021/acs.jafc.8b01113

Cheng, L.; Jin, Y.H.; Kim, I.H. Effects of Bacillus licheniformis derived-protease supplementation, alone or in combination, with valine to low protein diet on growth performance and carcass quality grade in growing-finishing pigs. *J. Appl. Anim. Res.* **2021**, *49*, 180–184. DOI: 10.1080/09712119.2021.1933494

Ma, W.; Mao, P.; Fan, W.; Zhu, Y.; Guo, L. Valine and isoleucine supplementation improve performance and serum biochemical concentrations in growing gilts fed low-protein diets. *Can. J. Anim. Sci.* **2019**, *99*, 921–928. DOI: 10.1139/cjas-2018-0169

Russell, L.E.; Kerr, B.J.; Easter, R.A. Limiting amino acids in an 11% crude protein corn-soybean meal diet for growing pigs. *J. Anim. Sci.* **1987**, *65*, 1266–1272. DOI: 10.2527/jas1987.6551266x

Mavromichalis, I.; Webel, D.M.; Emmert, J.L.; Moser, R.L.; Baker, D.H. Limiting order of amino acids in a low-protein corn-soybean meal-whey-based diet for nursery pigs. *J. Anim. Sci.* **1998**, *76*, 2833–2837. DOI: 10.2527/1998.76112833x

Liu, X.; Ma, W.; Zeng, X.; Xie, C.; Thacker, P.; Htoo, J.; Qiao, S.Y. Estimation of the standardized ileal digestible valine to lysine ratio required for 25-to 120-kg pigs fed low crude protein diets supplemented with crystalline amino acids. *J. Anim. Sci.* **2015**, *93*, 4761–4773. DOI: 10.2527/jas.2015-9308

Waguespack, A.; Bidner, T.; Payne, R.; Southern, L. Valine and isoleucine requirement of 20-to 45-kg pigs. *J. Anim. Sci.* **2012**, *90*, 2276–2284. DOI: 10.2527/jas.2011-4454

Powell, S.; Bidner, T.; Payne, R.; Southern, L. Growth performance of 20-to 50-kg pigs fed low-crude-protein diets supplemented with histidine, cystine, glycine, glutamic acid, or arginine. *J. Anim. Sci.* **2011**, *89*, 3643–3650. DOI: 10.2527/jas.2010-3757

Ren, M.; Zhang, S.; Zeng, X.; Liu, H.; Qiao, S. Branched-chain amino acids are beneficial to maintain growth performance and intestinal immune-related function in weaned piglets fed protein restricted diet. *Asian-Australas. J. Anim. Sci.* **2015**, *28*, 1742. DOI: 10.5713/ajas.14.0131

Sun, Y.; Wu, Z.; Li, W.; Zhang, C.; Sun, K.; Ji, Y.; Wang, B.; Jiao, N.; He, B.; Wang, W.; et al. Dietary L-leucine supplementation enhances intestinal development in suckling piglets. *Amino Acids* **2015**, *47*, 1517–1525. DOI: 10.1007/s00726-015-1985-2

Duan, Y.; Tan, B.; Li, J.; Liao, P.; Huang, B.; Li, F.; Xiao, H.; Liu, Y.; Yin, Y. Optimal branched-chain amino acid ratio improves cell proliferation and protein metabolism of porcine enterocytesin vivo and in vitro. *Nutr.* **2018**, *54*, 173–181. DOI: 10.1016/j.nut.2018.03.057

Zhang, S.; Qiao, S.; Ren, M.; Zeng, X.; Ma, X.; Wu, Z.; Thacker, P.; Wu, G. Supplementation with branched-chain amino acids to a low-protein diet regulates intestinal expression of amino acid and peptide transporters in weanling pigs. *Amino Acids* **2013**, *45*, 1191–1205. DOI: 10.1007/s00726-013-1577-y

Zheng, L.; Wei, H.; Cheng, C.; Xiang, Q.; Pang, J.; Peng, J. Supplementation of branched-chain amino acids to a reduced-protein diet improves growth performance in piglets: Involvement of increased feed intake and direct muscle growth-promoting effect. *Br. J. Nutr.* **2016**, *115*, 2236–2245. DOI: 10.1017/S0007114516000842

Tian, M.; Heng, J.; Song, H.; Shi, K.; Lin, X.; Chen, F.; Guan, W.; Zhang, S. Dietary branched-chain amino acids regulate food intake partly through intestinal and hypothalamic amino acid receptors in piglets. *J. Agric. Food Chem.* **2019**, *67*, 6809–6818. DOI: 10.1021/acs.jafc.9b02381

Zhou, H.; Yu, B.; Gao, J.; Htoo, J.K.; Chen, D. Regulation of intestinal health by branched-chain amino acids. *Anim. Sci. J.* **2018**, *89*, 3–11. DOI: 10.1111/asj.12937

Habibi, M.; Shili, C.N.; Sutton, J.; Goodarzi, P.; Pezeshki, A. Dietary branched-chain amino acids modulate the dynamics of calcium absorption and reabsorption in protein-restricted pigs. *J. Anim. Sci. Biotechnol.* **2022**, *13*, 1–16. DOI: 10.1186/s40104-021-00669-w

Kwon, W.B.; Soto, J.A.; Stein, H.H. Effects on nitrogen balance and metabolism of branched-chain amino acids by growing pigs of supplementing isoleucine and valine to diets with adequate or excess concentrations of dietary leucine. *J. Anim. Sci.* **2020**, *98*, skaa346. DOI: 10.1093/jas/skaa346

Yin, J.; Ma, J.; Li, Y.; Ma, X.; Chen, J.; Zhang, H.; Wu, X.; Li, F.; Liu, Z.; Li, T.; Yin, Y. Branched-chain amino acids, especially of leucine and valine, mediate the protein restricted response in a piglet model. *Food Funct.* **2020**, *11*, 1304–1311. DOI: 10.1039/C9FO01757G

Suryawan, A.; Jeyapalan, A.S.; Orellana, R.A.; Wilson, F.A.; Nguyen, H.V.; Davis, T.A. Leucine stimulates protein synthesis in skeletal muscle of neonatal pigs by enhancing mTORC1 activation. *Am. J. Physiol. Endocrinol. Metab.* **2008**, *295*, 868–875. DOI: 10.1152/ajpendo.90314.2008

Murgas Torrazza, R.; Suryawan, A.; Gazzaneo, M.C.; Orellana, R.A.; Frank, J.W.; Nguyen, H.V.; Fiorotto, M.L.; El-Kadi, S.; Davis, T.A. Leucine supplementation of a low-protein meal increases skeletal muscle and visceral tissue protein synthesis in neonatal pigs by stimulating mTOR-dependent translation initiation. *J. Nutr.* **2010**, *140*, 2145–2152. DOI: 10.3945/jn.110.128421

Yin, Y.; Yao, K.; Liu, Z.; Gong, M.; Ruan, Z.; Deng, D.; Tan, B.; Liu, Z.; Wu, G. Supplementing L-leucine to a low-protein diet increases tissue protein synthesis in weanling pigs. *Amino Acids* **2010**, *39*, 1477–1486. DOI: 10.1007/s00726-010-0612-5

Shili, C.N.; Broomhead, J.N.; Spring, S.C.; Lanahan, M.B.; Pezeshki, A. A novel corn-expressed phytase improves daily weight gain, protein efficiency ratio and nutrients digestibility and alters fecal microbiota in pigs fed with very low protein diets. Animals 2020, 10, 1926. DOI: 10.3390/ani10101926

Goodarzi, P.; Habibi, M.; Roberts, K.; Sutton, J.; Shili, C.N.; Lin, D.; Pezeshki, A. Dietary tryptophan supplementation alters fat and glucose metabolism in a low-birthweight piglet model. *Nutrients* **2021**, *13*, 2561. DOI: 10.3390/nu13082561

Kozich, J.J.; Westcott, S.L.; Baxter, N.T.; Highlander, S.K.; Schloss, P.D. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and environmental microbiology. *Appl. Environ. Microbiol.* **2013**, *79*, 5112–5120. DOI: 0.1128/AEM.01043-13

Cole, J.R.; Wang, Q.; Cardenas, E.; Fish, J.; Chai, B.; Farris, R.J.; Kulam-Syed-Mohideen, A.S.; McGarrell, D.M.; Marsh, T.; Garrity, G.M.; Tiedje, J.M. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Res.* **2009**, *37*, 141–145. DOI: 10.1093/nar/gkn879

51. Brosnan, J.T.; Brosnan, M.E. Branched-chain amino acids: enzyme and substrate regulation. *J Nutr.* **2006**, *136*, 207–211. DOI: 10.1093/jn/136.1.207S

Smith, T.K.; Austic, R.E. The branched-chain amino acid antagonism in chicks. *J. Nutr.* **1978**, *108*, 1180–1191. DOI: 10.1093/jn/108.7.1180

Xiao, F.; Guo, F. Impacts of essential amino acids on energy balance. *Mol Metab.* **2022**, *57*, 101393. DOI: 10.1016/j.molmet.2021.101393

Wiltafsky, M.K.; Schmidtlein, B.; Roth, F.X. Estimates of the optimum dietary ratio of standardized ileal digestible valine to lysine for eight to twenty-five kilograms of body weight pigs. *J. Anim. Sci.* **2009**, *87*, 2544–2553. DOI: 10.2527/jas.2008-1221

Salmon, W.D. The significance of amino acid imbalance in nutrition. *Am J Clin Nutr.* **1958**, *6*, 487–494. DOI: 10.1093/ajcn/6.5.487

56. Fan, P.; Liu, P.; Song, P.; Chen, X.; Ma, X. Moderate dietary protein restriction alters the composition of gut microbiota and improves ileal barrier function in adult pig model. *Sci. Rep.* **2017**, *7*, 43412. DOI: 10.1038/srep43412

Pajarillo, E.A.B.; Chae, J.P.; Balolong, M.P.; Kim, H.B.; Kang, D.K. Assessment of fecal bacterial diversity among healthy piglets during the weaning transition. *J. Gen. Appl. Microbiol.* **2014**, *60*, 140–146. DOI: 10.2323/jgam.60.140

Kim, H.B.; Borewicz, K.; White, B.A.; Singer, R.S.; Sreevatsan, S.; Tu, Z.J.; Isaacson, R.E. Longitudinal investigation of the age-related bacterial diversity in the feces of commercial pigs. *Vet. Microbiol.* **2011**, *153*, 124–133. DOI: 10.1016/j.vetmic.2011.05.021

McCormack, U.M.; Curião, T.; Buzoianu, S.G.; Prieto, M.L.; Ryan, T.; Varley, P.; Crispie, F.; Magowan, E.; Metzler-Zebeli, B.U.; Berry, D.; O'Sullivan, O. Exploring a possible link between the intestinal microbiota and feed efficiency in pigs. *Appl. Environ. Microbiol.* **2017**, *83*, e00380–17. DOI: 10.1128/AEM.00380-17

Flint, H.J.; Bayer, E.A. Plant cell wall breakdown by anaerobic microorganisms from the mammalian digestive tract. *Ann. N.Y. Acad. Sci.* **2008**, *1225*, 280–288. DOI: 10.1196/annals.1419.022

Guevarra, R.B.; Hong, S.H.; Cho, J.H.; Kim, B.R.; Shin, J.; Lee, J.H.; Kang, B.N.; Kim, Y.H.; Wattanaphansak, S.; Isaacson, R.E.; Song, M. The dynamics of the piglet gut microbiome during the weaning transition in association with health and nutrition. *J. Anim. Sci. Biotechnol.* **2018**, *9*, 1–9. DOI: 10.1186/s40104-018-0269-6

Frese, S.A.; Parker, K.; Calvert, C.C.; Mills, D.A. Diet shapes the gut microbiome of pigs during nursing and weaning. *Microbiome* **2015**, *3*, 1–10. DOI: 10.1186/s40168-015-0091-8

Koo, B.; Kim, J.W.; Nyachoti, C.M. Nutrient and energy digestibility, and microbial metabolites in weaned pigs fed diets containing Lactobacillus–fermented wheat. *Anim. Feed Sci. Technol.* **2018**, *241*, 27–37. DOI: 10.1016/j.anifeedsci.2018.04.007

Marounek, M.I.L.A.N.; Fliegrova, K.A.T.E.R.I.N.A.; Bartos, S.T.A.N.I.S.L.A.V. Metabolism and some characteristics of ruminal strains of Megasphaera elsdenii. *Appl Environ Microbiol.* **1989**, *55*, 1570– 1573. DOI: 10.1128/aem.55.6.1570-1573.1989

Quan, J.; Cai, G.; Yang, M.; Zeng, Z.; Ding, R.; Wang, X.; Zhuang, Z.; Zhou, S.; Li, S.; Yang, H.; Li, Z. Exploring the fecal microbial composition and metagenomic functional capacities associated with feed efficiency in commercial DLY pigs. *Front. Microbiol.* **2019**, *10*, 52. DOI: 10.3389/fmicb.2019.00052

Yang, H.; Yang, M.; Fang, S.; Huang, X.; He, M.; Ke, S.; Gao, J.; Wu, J.; Zhou, Y.; Fu, H.; Chen, C. Evaluating the profound effect of gut microbiome on host appetite in pigs. *BMC Microbiol.* **2018**, *18*, 1–10. DOI: 10.1186/s12866-018-1364-8

Rattigan, R.; Sweeney, T.; Maher, S.; Ryan, M.T.; Thornton, K.; O'Doherty, J.V. Effects of reducing dietary crude protein concentration and supplementation with either laminarin or zinc

oxide on the growth performance and intestinal health of newly weaned pigs. *Anim. Feed Sci. Technol.* **2020**, *270*, 114693. DOI: 10.1016/j.anifeedsci.2020.114693

Christensen, H.; Bossé, J.; Angen, Ø.; Nørskov-Lauritsen, N.; Bisgaard, M. Immunological and molecular techniques used for determination of serotypes in Pasteurellaceae. In *Book Immunological Methods in Microbiology*, 1st ed.; Pavia, C.S., Gurtler, V., Eds.; Academic Press: London, United Kingdom, 2020; 47, pp. 117–149. DOI: 10.1016/bs.mim.2020.01.002

Stetsko, T.I. Antibiotic resistance of bacteria of the family pasteurellaceae, pathogens of respiratory infections of cattle and pigs. *Sci. Tech. Bull. State Sci. Res. Con. Ins. Vet. Med. Pro. Fodder Add. Ins. Anim. Bio.* **2021**, *22*, 197–208. DOI: 10.36359/scivp.2021-22-1.24

Dou, S.; Gadonna-Widehem, P.; Rome, V.; Hamoudi, D.; Rhazi, L.; Lakhal, L.; Larcher, T.; Bahi-Jaber, N.; Pinon-Quintana, A.; Guyonvarch, A.; Huërou-Luron, I.L. Characterisation of early-life fecal microbiota in susceptible and healthy pigs to post-weaning diarrhoea. *PLoS One.* **2017**, *12*, e0169851. DOI: 10.1371/journal.pone.0169851

Allen-Vercoe, E.; Jobin, C. Fusobacterium and Enterobacteriaceae: Important players for CRC? *Immunol. Lett.* **2014**, *162*, 54–61. DOI: 10.1016/j.imlet.2014.05.014

Guevarra, R.B.; Lee, J.H.; Lee, S.H.; Seok, M.J.; Kim, D.W.; Kang, B.N.; Johnson, T.J.; Isaacson, R.E.; Kim, H.B. Piglet gut microbial shifts early in life: causes and effects. *J. Anim. Sci. Biotechnol.* **2019**, *10*, 1–10. DOI: 10.1186/s40104-018-0308-3

Gresse, R.; Chaucheyras-Durand, F.; Fleury, M.A.; Van de Wiele, T.; Forano, E.; Blanquet-Diot, S. Gut microbiota dysbiosis in postweaning piglets: understanding the keys to health. *Trends Microbiol.* **2017**, *25*, 851–873. DOI: 10.1016/j.tim.2017.05.004

Rhouma, M.; Fairbrother, J.M.; Beaudry, F.; Letellier, A. Post weaning diarrhea in pigs: risk factors and non-colistin-based control strategies. *Acta Vet. Scand.* **2017**, *59*, 1–19. DOI: 10.1186/s13028-017-0299-7

Binda, C.; Lopetuso, L.R.; Rizzatti, G.; Gibiino, G.; Cennamo, V.; Gasbarrini, A. Actinobacteria: a relevant minority for the maintenance of gut homeostasis. *Dig. Liver Dis.* **2018**, *50*, 421–428. DOI: 10.1016/j.dld.2018.02.012

Bibbò, S.; Abbondio, M.; Sau, R.; Tanca, A.; Pira, G.; Errigo, A.; Manetti, R.; Pes, G.M.; Dore, M.P.; Uzzau, S. Fecal microbiota signatures in celiac disease patients with poly-autoimmunity. *Front. cell. infect.* **2020**, *10*, 349. DOI: 10.3389/fcimb.2020.00349

Mei, L.; Zhou, J.; Su, Y.; Mao, K.; Wu, J.; Zhu, C.; He, L.; Cui, Y. Gut microbiota composition and functional prediction in diarrhea-predominant irritable bowel syndrome. *BMC Gastroenterol.* **2021**, *21*, 1–12. DOI: 10.1186/s12876-021-01693-w

Tan, G.Y.; Liu, T. Rational synthetic pathway refactoring of natural products biosynthesis in actinobacteria. *Metab. Eng.* **2017**, *39*, 228–236. DOI: 10.1016/j.ymben.2016.12.006

Zeyner, A.; Boldt, E. Effects of a probiotic Enterococcus faecium strain supplemented from birth to weaning on diarrhoea patterns and performance of piglets. *J. Anim. Physiol. Anim. Nutr.* **2006**, *90*, 25–31. DOI: 10.1111/j.1439-0396.2005.00615.x

Bednorz, C.; Guenther, S.; Oelgeschläger, K.; Kinnemann, B.; Pieper, R.; Hartmann, S.; Tedin, K.; Semmler, T.; Neumann, K.; Schierack, P.; Bethe, A. Feeding the probiotic Enterococcus faecium strain NCIMB 10415 to piglets specifically reduces the number of Escherichia coli pathotypes that adhere to the gut mucosa. *Appl. Environ. Microbiol.* **2013**, *79*, 7896–7904. DOI: 10.1128/AEM.03138-13

Wang, Y.B.; Du, W.; Fu, A.K.; Zhang, X.P.; Huang, Y.; Lee, K.H.; Yu, K.; Li, W.F.; Li, Y.L. Intestinal microbiota and oral administration of Enterococcus faecium associated with the growth performance of new-born piglets. *Benef. Microbes.* **2016**, *7*, 529–538. DOI: 10.3920/BM2015.0099

Strompfova, V.; Marciňáková, M.; Simonová, M.; Gancarčíková, S.; Jonecová, Z.; Sciranková, Ľ.; Koščová, J.; Buleca, V.; Čobanová, K.; Lauková, A. Enterococcus faecium EK13—an enterocin A-producing strain with probiotic character and its effect in piglets. *Anaerobe* **2006**, *12*, 242–248. DOI: 10.1016/j.anaerobe.2006.09.003

Seddon, B.; McHugh, R.C.; Schmitt, A. Brevibacillus brevis-a novel candidate biocontrol agent with broad-spectrum antifungal activity. In The BCPC Conference: Pests and diseases, Volume 2. Proceedings of an international conference held at the Brighton Hilton Metropole Hotel, Brighton, UK, 13-16 November 2000, pp. 563–570. British Crop Protection Council, 2000.

Che, J.; Ye, S.; Liu, B.; Deng, Y.; Chen, Q.; Ge, C.; Liu, G.; Wang, J. Effects of Brevibacillus brevis FJAT-1501-BPA on growth performance, faecal microflora, faecal enzyme activities and blood parameters of weaned piglets. *Anton. Leeuw. Int. J. Gen.* **2016**, *109*, 1545–1553. DOI: 10.1007/s10482-016-0756-8

Zhu, L.; Liao, R.; Tu, W.; Lu, Y.; Cai X. Pyrodextrin enhances intestinal function through changing the intestinal microbiota composition and metabolism in early weaned piglets. *Appl Microbiol Biotechnol.* **2020**, *104*, 4141–4154. DOI: 10.1007/s00253-020-10419-z.

Webb, J.; Broomfield, M.; Jones, S.; Donovan, B. Ammonia and odour emissions from UK pig farms and nitrogen leaching from outdoor pig production. A review. *Sci. Total Environ.* **2014**, *470*, 865–875. DOI: 10.1016/j.scitotenv.2013.09.091.

Deng, D.; Huang, R.; Li, T.; Wu, G.; Xie, M.; Tang, Z.; Kang, P.; Zhang, Y.; Fan, M.; Kong, X. Nitrogen balance in barrows fed low-protein diets supplemented with essential amino acids. *Livest. Sci.* **2007**, *109*, 220–223. DOI: 10.1016/j.livsci.2007.01.122.

He, L.; Wu, L.; Xu, Z.; Li, T.; Yao, K.; Cui, Z.; Yin, Y.; Wu, G. Low-protein diets affect ileal amino acid digestibility and gene expression of digestive enzymes in growing and finishing pigs. *Amino acids* **2016**, *48*, 21–30. DOI: 10.1007/s00726-015-2059-1.

Portejoie, S.; Dourmad, J.-Y.; Martinez, J.; Lebreton, Y. Effect of lowering dietary crude protein on nitrogen excretion, manure composition and ammonia emission from fattening pigs. *Livest. Prod. Sci.* **2004**, *91*, 45–55. DOI: 10.1016/j.livprodsci.2004.06.013.

Nyachoti, C.; Omogbenigun, F.; Rademacher, M.; Blank, G. Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. *J. Anim. Sci.* **2006**, *84*, 125–134. DOI: 10.2527/2006.841125x.

Wu, L.; Liao, P.; He, Q.; Tan, B.; Guo, F.; Tang, M.; Li, T. Chronic feeding with protein-restricted diets affect ileal amino acid digestibility and the expression of nutrient-sensing, hormone secretion, gastrointestinal digestive enzyme, and nutrient transporter genes in young weaned pigs. *Oncotarget* **2018**, *5*, DOI: Retrieved from https://www.oncotarget.com/article/24093/text/.

Spring, S.; Premathilake, H.; DeSilva, U.; Shili, C.; Carter, S.; Pezeshki, A. Low protein-high carbohydrate diets alter energy balance, gut microbiota composition and blood metabolomics profile in young pigs. *Sci. Rep.* **2020**, *10*, 1–15. DOI: 10.1038/s41598-020-60150-y.

Luo, Z.; Li, C.; Cheng, Y.; Hang, S.; Zhu, W. Effects of low dietary protein on the metabolites and microbial communities in the caecal digesta of piglets. *Arch. Anim. Nutr.* **2015**, *69*, 212–226. DOI: 10.1080/1745039X.2015.1034521.

Spring, S.; Premathilake, H.; Bradway, C.; Shili, C.; DeSilva, U.; Carter, S.; Pezeshki, A. Effect of very low-protein diets supplemented with branched-chain amino acids on energy balance, plasma metabolomics and fecal microbiome of pigs. *Sci. Rep.* **2020**, *10*, 1–16. DOI: 10.1038/s41598-020-72816-8.

Habibi, M.; Shili, C.N.; Sutton, J.; Goodarzi, P.; Pezeshki, A. Dietary branched-chain amino acids modulate the dynamics of calcium absorption and reabsorption in protein-restricted pigs. *J. Anim. Sci. Biotechnol.* **2022**, *13*, 1–16. DOI: 10.1186/s40104-021-00669-w.

Habibi, M.; Goodarzi, P.; Shili, C.N.; Sutton, J.; Wileman, C.M.; Kim, D.M.; Lin, D.; Pezeshki, A. A Mixture of Valine and Isoleucine Restores the Growth of Protein-Restricted Pigs Likely through Improved Gut Development, Hepatic IGF-1 Pathway, and Plasma Metabolomic Profile. *Int. J. Mol. Sci.* **2022**, *23*, 3300. DOI: 10.3390/ijms23063300.

Kwon, W.B.; Soto, J.A.; Stein, H.H. Effects on nitrogen balance and metabolism of branched-chain amino acids by growing pigs of supplementing isoleucine and valine to diets with adequate or excess concentrations of dietary leucine. *J. Anim. Sci.* **2020**, *98*, skaa346. DOI: 10.1093/jas/skaa346.

Liu, X.; Ma, W.; Zeng, X.; Xie, C.; Thacker, P.; Htoo, J.; Qiao, S. Estimation of the standardized ileal digestible valine to lysine ratio required for 25-to 120-kilogram pigs fed low crude protein diets supplemented with crystalline amino acids. *J. Anim. Sci.* **2015**, *93*, 4761–4773. DOI: 10.2527/jas.2015-9308.

Zhang, X.; Liu, X.; Jia, H.; He, P.; Mao, X.; Qiao, S.; Zeng, X. Valine supplementation in a reduced protein diet regulates growth performance partially through modulation of plasma amino acids profile, metabolic responses, endocrine, and neural factors in piglets. *J. Agric. Food Chem.* **2018**, *66*, 3161–3168. DOI: 10.1021/acs.jafc.8b01113.

Parr, T.; Kerr, B.; Baker, D. Isoleucine requirement of growing (25 to 45 kg) pigs. *J. Anim. Sci.* **2003**, *81*, 745–752. DOI: 10.2527/2003.813745x.

Lordelo, M.; Gaspar, A.; Le Bellego, L.; Freire, J. Isoleucine and valine supplementation of a low-protein corn-wheat-soybean meal-based diet for piglets: growth performance and nitrogen balance. *J. Anim. Sci.* **2008**, *86*, 2936–2941. DOI: 10.2527/jas.2007-0222.

Figueroa, J.; Lewis, A.; Miller, P.; Fischer, R.; Gómez, R.; Diedrichsen, R. Nitrogen metabolism and growth performance of gilts fed standard corn-soybean meal diets or low-crude protein, amino acid-supplemented diets. *J. Anim. Sci.* **2002**, *80*, 2911–2919. DOI: 10.2527/2002.80112911x.

Ren, M.; Zhang, S.; Zeng, X.; Liu, H.; Qiao, S. Branched-chain amino acids are beneficial to maintain growth performance and intestinal immune-related function in weaned piglets fed protein restricted diet. *Asian-Australasian J. Anim. Sci. s* **2015**, *28*, 1742. DOI: 10.5713/ajas.14.0131.

Ma, W.; Mao, P.; Fan, W.; Zhu, Y.; Guo, L. Valine and isoleucine supplementation improve performance and serum biochemical concentrations in growing gilts fed low-protein diets. *Canadian J. Anim. Sci.* **2019**, *99*, 921–928. DOI: 10.1139/cjas-2018-0169.

Echenique, M.; Bistrian, B.; Moldawer, L.; Palombo, J.; Miller, M.; Blackburn, G. Improvement in amino acid use in the critically ill patient with parenteral formulas enriched with branched chain amino acids. *Surg. Gynecol. Obstet.* **1984**, *159*, 233–241.

Wang, X.-Y.; Li, N.; Gu, J.; Li, W.-Q.; Li, J.-S. The effects of the formula of amino acids enriched BCAA on nutritional support in traumatic patients. *World J. Gastroenterol.* **2003**, *9*, 599. DOI: 10.3748/wjg.v9.i3.599.

Tamanna, N.; Mahmood, N. Emerging roles of branched-chain amino acid supplementation in human diseases. *Int Sch Res Notices* **2014**, *2014*, 235619. DOI: 10.1155/2014/235619.

Liu, K.; Liu, Y.; Liu, S.; Xu, M.; Yu, Z.; Wang, X.; Cao, Y.; Yao, J. Relationships between leucine and the pancreatic exocrine function for improving starch digestibility in ruminants. *J. Dairy Sci.* **2015**, *98*, 2576–2582. DOI: 10.3168/jds.2014-8404.

Zhao, J.; Liu, Y.; Jiang, J.; Wu, P.; Chen, G.; Jiang, W.; Li, S.; Tang, L.; Kuang, S.; Feng, L. Effects of dietary isoleucine on growth, the digestion and absorption capacity and gene expression in hepatopancreas and intestine of juvenile Jian carp (Cyprinus carpio var. Jian). *Aquaculture* **2012**, *368*, 117–128. DOI: 10.1016/j.aquaculture.2012.09.019.

Dong, M.; Feng, L.; Kuang, S.Y.; Liu, Y.; Jiang, J.; Hu, K.; Jiang, W.D.; Li, S.H.; Tang, L.; Zhou, X.Q. Growth, body composition, intestinal enzyme activities and microflora of juvenile Jian carp (Cyprinus carpio var. Jian) fed graded levels of dietary valine. *Aquac. Nutr.* **2013**, *19*, 1–14. DOI: 10.1111/j.1365-2095.2011.00926.x.

Guo, L.; Yao, J.; Zheng, C.; Tian, H.; Liu, Y.; Liu, S.; Cai, C.; Xu, X.; Cao, Y. Leucine regulates α-amylase and trypsin synthesis in dairy calf pancreatic tissue in vitro via the

mammalian target of rapamycin signalling pathway. *Animal* **2019**, *13*, 1899–1906. DOI: 10.1017/S175173111800368.

Zhang, S.; Qiao, S.; Ren, M.; Zeng, X.; Ma, X.; Wu, Z.; Thacker, P.; Wu, G. Supplementation with branched-chain amino acids to a low-protein diet regulates intestinal expression of amino acid and peptide transporters in weanling pigs. *Amino acids* **2013**, *45*, 1191–1205. DOI: 10.1007/s00726-013-1577-y.

Sun, Y.; Wu, Z.; Li, W.; Zhang, C.; Sun, K.; Ji, Y.; Wang, B.; Jiao, N.; He, B.; Wang, W. Dietary L-leucine supplementation enhances intestinal development in suckling piglets. *Amino Acids* **2015**, *47*, 1517–1525. DOI: 10.1007/s00726-015-1985-2.

Zhou, H.; Yu, B.; Gao, J.; Htoo, J.K.; Chen, D. Regulation of intestinal health by branched-chain amino acids. *Anim. Sci. J.* **2018**, *89*, 3–11. DOI: 10.1111/asj.12937.

Sciacovelli, M.; Dugourd, A.; Jimenez, L.V.; Yang, M.; Nikitopoulou, E.; Costa, A.S.; Tronci, L.; Caraffini, V.; Rodrigues, P.; Schmidt, C. Nitrogen partitioning between branched-chain amino acids and urea cycle enzymes sustains renal cancer progression. *BioRxiv* **2021**, DOI: 10.1101/2021.09.17.460635.

Gumus Balikcioglu, P.; Ramaker, M.E.; Mason, K.A.; Huffman, K.M.; Johnson, J.L.; Ilkayeva, O.; Muehlbauer, M.J.; Freemark, M.; Kraus, W.E. Branched-Chain Amino Acid Catabolism and Cardiopulmonary Function Following Acute Maximal Exercise Testing in Adolescents. *Front. cardiovasc. med.* **2021**, *8*, 721354. DOI: 10.3389/fcvm.2021.721354.

Wu, G. Amino acids: metabolism, functions, and nutrition. *Amino acids* **2009**, *37*, 1–17. DOI: 10.1007/s00726-009-0269-0.

Habibi, M.; Shili, C.; Sutton, J.; Goodarzi, P.; Maylem, E.R.; Spicer, L.; Pezeshki, A. Branched-chain amino acids partially recover the reduced growth of pigs fed with protein-restricted diets through both central and peripheral factors. *Anim. Nutr.* **2021**, *7*, 868–882. DOI: 10.1016/j.aninu.2021.02.002.

Arakawa, M.; Masaki, T.; Nishimura, J.; Seike, M.; Yoshimatsu, H. The effects of branched-chain amino acid granules on the accumulation of tissue triglycerides and uncoupling proteins in diet-induced obese mice. *Endocr. J.* **2011**, *58*, 161–170. DOI: 10.1507/endocrj.k10e-221.

Nishimura, J.; Masaki, T.; Arakawa, M.; Seike, M.; Yoshimatsu, H. Isoleucine prevents the accumulation of tissue triglycerides and upregulates the expression of PPARα and uncoupling protein in diet-induced obese mice. *J. Nutr.* **2010**, *140*, 496–500. DOI: 10.3945/jn.109.108977.

Ma, Q.; Zhou, X.; Hu, L.; Chen, J.; Zhu, J.; Shan, A. Leucine and isoleucine have similar effects on reducing lipid accumulation, improving insulin sensitivity and increasing the browning of WAT in high-fat diet-induced obese mice. *Food function* **2020**, *11*, 2279–2290. DOI: 10.1039/C9FO03084K.

Zhang, F.; Zhao, S.; Yan, W.; Xia, Y.; Chen, X.; Wang, W.; Zhang, J.; Gao, C.; Peng, C.; Yan, F. Branched chain amino acids cause liver injury in obese/diabetic mice by promoting adipocyte lipolysis and inhibiting hepatic autophagy. *EBioMedicine* **2016**, *13*, 157–167. DOI: 10.1016/j.ebiom.2016.10.013.

Ma, Q.; Hu, L.; Zhu, J.; Chen, J.; Wang, Z.; Yue, Z.; Qiu, M.; Shan, A. Valine supplementation does not reduce lipid accumulation and improve insulin sensitivity in mice fed high-fat diet. *ACS omega* **2020**, *5*, 30937–30945. DOI: 10.1021/acsomega.0c03707.

Zhang, H.; Xiang, L.; Huo, M.; Wu, Y.; Yu, M.; Lau, C.W.; Tian, D.; Gou, L.; Huang, Y.; Luo, J.Y. Branched-chain amino acid supplementation impairs insulin sensitivity and promotes lipogenesis during exercise in diet-induced obese mice. *Obesity* **2022**, DOI: 10.1002/oby.23394.

Guo, F.; Cavener, D.R. The GCN2 eIF2α kinase regulates fatty-acid homeostasis in the liver during deprivation of an essential amino acid. *Cell metabolism* **2007**, *5*, 103–114. DOI: 10.1016/j.cmet.2007.01.001.

Cheng, Y.; Meng, Q.; Wang, C.; Li, H.; Huang, Z.; Chen, S.; Xiao, F.; Guo, F. Leucine deprivation decreases fat mass by stimulation of lipolysis in white adipose tissue and upregulation of uncoupling protein 1 (UCP1) in brown adipose tissue. *Diabetes* **2010**, *59*, 17–25. DOI: 10.2337/db09-0929.

Du, Y.; Meng, Q.; Zhang, Q.; Guo, F. Isoleucine or valine deprivation stimulates fat loss via increasing energy expenditure and regulating lipid metabolism in WAT. *Amino acids* **2012**, *43*, 725–734. DOI: 10.1007/s00726-011-1123-8.

Bai, J.; Greene, E.; Li, W.; Kidd, M.T.; Dridi, S. Branched-chain amino acids modulate the expression of hepatic fatty acid metabolism-related genes in female broiler chickens. *Molecular nutrition food research* **2015**, *59*, 1171–1181. DOI: 10.1002/mnfr.201400918.

McGarrah, R.W.; Zhang, G.-F.; Christopher, B.A.; Deleye, Y.; Walejko, J.M.; Page, S.; Ilkayeva, O.; White, P.J.; Newgard, C.B. Dietary branched-chain amino acid restriction alters fuel selection and reduces triglyceride stores in hearts of Zucker fatty rats. *American Journal of Physiology-Endocrinology Metabolism* **2020**, *318*, E216–E223. DOI: 10.1152/ajpendo.00334.2019.

Miller, E.; Ullrey, D. The pig as a model for human nutrition. *Annual review of nutrition* **1987**, *7*, 361–382. DOI: 10.1146/annurev.nu.07.070187.002045.

Baker, D.H. Animal models in nutrition research. *J. Nutr.* **2008**, *138*, 391–396. DOI: 10.1093/jn/138.2.391.

Ball, R.O.; Urschel, K.L.; Pencharz, P.B. Nutritional consequences of interspecies differences in arginine and lysine metabolism. *J. Nutr.* **2007**, *137*, 1626S–1641S. DOI: 10.1093/jn/137.6.1626S.

Riedijk, M.A.; Stoll, B.; Chacko, S.; Schierbeek, H.; Sunehag, A.L.; van Goudoever, J.B.; Burrin, D.G. Methionine transmethylation and transsulfuration in the piglet gastrointestinal tract. *Proc. Natl. Acad. Sci.* **2007**, *104*, 3408–3413. DOI: 10.1073/pnas.0607965104.

Bellinger, D.A.; Merricks, E.P.; Nichols, T.C. Swine models of type 2 diabetes mellitus: insulin resistance, glucose tolerance, and cardiovascular complications. *ILAR J.* **2006**, *47*, 243–258. DOI: 10.1093/ilar.47.3.243.

Harwood Jr, H.J.; Listrani, P.; Wagner, J.D.J.J.O.D.S.; technology. Nonhuman primates and other animal models in diabetes research. *J. Diabetes* **2012**, *6*, 503–514. DOI: 10.1177/1932296812006003.

NRC. *Nutrient Requirements of Swine: Eleventh Revised Edition*; The National Academies Press: Washington, DC, USA, 2012.

Nørgaard, J.; Fernández, J. Isoleucine and valine supplementation of crude protein-reduced diets for pigs aged 5–8 weeks. *Anim. Feed Sci. Technol.* **2009**, *154*, 248–253. DOI: 10.1016/j.anifeedsci.2009.08.010.

Goodarzi, P.; Habibi, M.; Roberts, K.; Sutton, J.; Shili, C.N.; Lin, D.; Pezeshki, A. Dietary tryptophan supplementation alters fat and glucose metabolism in a low-birthweight piglet model. *Nutrients* **2021**, *13*, 2561. DOI: 10.3390/nu13082561.

Shili, C.N.; Broomhead, J.N.; Spring, S.C.; Lanahan, M.B.; Pezeshki, A. A novel corn-expressed phytase improves daily weight gain, protein efficiency ratio and nutrients digestibility and alters fecal microbiota in pigs fed with very low protein diets. *Animals* **2020**, *10*, 1926. DOI: 10.3390/ani10101926.

Pezeshki, A.; Muench, G.; Chelikani, P. Expression of peptide YY, proglucagon, neuropeptide Y receptor Y2, and glucagon-like peptide-1 receptor in bovine peripheral tissues. *J. Dairy Sci.* **2012**, *95*, 5089–5094. DOI: 10.3168/jds.2011-5311.

Shili, C.N.; Habibi, M.; Sutton, J.; Barnes, J.; Burch-Konda, J.; Pezeshki, A. Effect of a phytogenic water additive on growth performance, blood metabolites and gene expression of amino acid transporters in nursery pigs fed with low-protein/high-carbohydrate diets. *Animals* **2021**, *11*, 555. DOI: 10.3390/ani11020555.

Zhou, X.; Wan, D.; Zhang, Y.; Zhang, Y.; Long, C.; Chen, S.; He, L.; Tan, B.; Wu, X.; Yin, Y. Diurnal variations in polyunsaturated fatty acid contents and expression of genes involved in their de novo synthesis in pigs. *Biochem. Biophys. Res. Commun.* **2017**, *483*, 430–434. DOI: 10.1016/j.bbrc.2016.12.126.

Espinosa, C.D.; Fry, R.S.; Kocher, M.E.; Stein, H.H. Effects of copper hydroxychloride on growth performance and abundance of genes involved in lipid metabolism of growing pigs. *J. Anim. Sci.* **2020**, *98*, skz369. DOI: 10.1093/jas/skz369.

Chen, G.; Zhang, J.; Zhang, Y.; Liao, P.; Li, T.; Chen, L.; Yin, Y.; Wang, J.; Wu, G. Oral MSG administration alters hepatic expression of genes for lipid and nitrogen metabolism in suckling piglets. *Amino Acids* **2014**, *46,* 245–250. DOI: 10.1007/s00726-013-1615-9.

Yin, J.; Liu, M.; Ren, W.; Duan, J.; Yang, G.; Zhao, Y.; Fang, R.; Chen, L.; Li, T.; Yin, Y. Effects of dietary supplementation with glutamate and aspartate on diquat-induced oxidative stress in piglets. *PloS one* **2015**, *10,* e0122893. DOI: 10.1371/journal.pone.0122893.

Zuo, F.; Gu, Q.; Li, S.; Wei, H.; Peng, J. Effects of different methionine sources on methionine metabolism in the IPEC-J2 cells. *Biomed Res. Int.* **2019**, *2019*, DOI: 10.1155/2019/5464906.

Duran-Montgé, P.; Theil, P.; Lauridsen, C.; Esteve-Garcia, E. Dietary fat source affects metabolism of fatty acids in pigs as evaluated by altered expression of lipogenic genes in liver and adipose tissues. *Animal* **2009**, *3*, 535–542. DOI: 10.1017/S1751731108003686.

Kang, P.; Liu, Y.; Zhu, H.; Zhang, J.; Shi, H.; Li, S.; Pi, D.; Leng, W.; Wang, X.; Wu, H. The effect of dietary asparagine supplementation on energy metabolism in liver of weaning pigs when challenged with lipopolysaccharide. *Asian-Australasian J. Anim. Sci. s* **2018**, *31,* 548.

Pezeshki, A.; Chelikani, P.K. Effects of Roux-en-Y gastric bypass and ileal transposition surgeries on glucose and lipid metabolism in skeletal muscle and liver. *Surg. Obes. Relat. Dis.* **2014**, *10,* 217–228. DOI: 10.1016/j.soard.2013.09.017.

Figueroa, J.; Lewis, A.; Miller, P.; Fischer, R.; Diedrichsen, R. Growth, carcass traits, and plasma amino acid concentrations of gilts fed low-protein diets supplemented with amino acids including histidine, isoleucine, and valine. *J. Anim. Sci.* **2003**, *81,* 1529–1537. DOI: 10.2527/2003.8161529x.

Russell, L.E.; Kerr, B.J.; Easter, R.A. Limiting amino acids in an 11% crude protein corn-soybean meal diet for growing pigs. *J. Anim. Sci.* **1987**, *65*, 1266–1272. DOI: 10.2527/jas1987.6551266x.

Zheng, L.; Wei, H.; Cheng, C.; Xiang, Q.; Pang, J.; Peng, J. Supplementation of branched-chain amino acids to a reduced-protein diet improves growth performance in piglets: involvement of increased feed intake and direct muscle growth-promoting effect. *Br. J. Nutr.* **2016**, *115*, 2236–2245. DOI: 10.1017/S0007114516000842.

Tian, M.; Heng, J.; Song, H.; Shi, K.; Lin, X.; Chen, F.; Guan, W.; Zhang, S. Dietary branched-chain amino acids regulate food intake partly through intestinal and hypothalamic amino acid receptors in piglets. *J. Agric. Food Chem.* **2019**, *67*, 6809–6818. DOI: 10.1021/acs.jafc.9b02381.

Salmon, W.D. The significance of amino acid imbalance in nutrition. *Am. J. Clin. Nutr.* **1958**, *6*, 487–494. DOI: 10.1093/ajcn/6.5.487.

Xiao, F.; Guo, F. Impacts of essential amino acids on energy balance. *Mol. Metab.* **2021**, 101393. DOI: 10.1016/j.molmet.2021.101393.

Arrieta-Cruz, I.; Su, Y.; Gutiérrez-Juárez, R. Suppression of endogenous glucose production by isoleucine and valine and impact of diet composition. *Nutrients* **2016**, *8*, 79. DOI: 10.3390/nu8020079.

Elovaris, R.A.; Bitarafan, V.; Agah, S.; Ullrich, S.S.; Lange, K.; Horowitz, M.; Feinle-Bisset, C. Comparative Effects of the Branched-Chain Amino Acids, Leucine, Isoleucine and Valine, on Gastric Emptying, Plasma Glucose, C-Peptide and Glucagon in Healthy Men. *Nutrients* **2021**, *13*, 1613. DOI: 10.3390/nu13051613.

Ullrich, S.S.; Fitzgerald, P.C.; Schober, G.; Steinert, R.E.; Horowitz, M.; Feinle-Bisset, C. Intragastric administration of leucine or isoleucine lowers the blood glucose response to a mixed-nutrient drink by different mechanisms in healthy, lean volunteers. *Am. J. Clin. Nutr.* **2016**, *104*, 1274–1284. DOI: 10.3945/ajcn.116.140640.

Nishitani, S.; Takehana, K.; Fujitani, S.; onaka, I. Branched-chain amino acids improve glucose metabolism in rats with liver cirrhosis. *Am J Physiol Gastrointest Liver Physiol* **2005**, *288*, G1292–G1300. DOI: 10.1152/ajpgi.00510.2003.

Doi, M.; Yamaoka, I.; Fukunaga, T.; Nakayama, M. Isoleucine, a potent plasma glucose-lowering amino acid, stimulates glucose uptake in C2C12 myotubes. *Biochem. Biophys. Res. Commun.* **2003**, *312*, 1111–1117. DOI: 10.1016/j.bbrc.2003.11.039.

Doi, M.; Yamaoka, I.; Nakayama, M.; Sugahara, K.; Yoshizawa, F. Hypoglycemic effect of isoleucine involves increased muscle glucose uptake and whole body glucose oxidation and decreased hepatic gluconeogenesis. *Am. J. Physiol. Endocrinol. Metab.* **2007**, *292*, E1683–E1693. DOI: 10.1152/ajpendo.00609.2006.

Nuttall, F.Q.; Schweim, K.; Gannon, M.C. Effect of orally administered isoleucine with and without glucose on insulin, glucagon and glucose concentrations in non-diabetic subjects. *E Spen Eur E J Clin Nutr Metab* **2008**, *3*, e152–e158. DOI: 10.1016/j.eclnm.2008.05.001.

Floyd, J.; Fajans, S.S.; Conn, J.W.; Knopf, R.F.; Rull, J. Stimulation of insulin secretion by amino acids. *J. Clin. Investig.* **1966**, *45*, 1487–1502. DOI: 10.1172/JCI105456.

Wang, H.; Wang, X.; Zhao, J.; Jiao, H.; Lin, H. Low protein diet supplemented with crystalline amino acids suppressing appetite and apo-lipoprotein synthesis in laying hens. *Anim. Feed Sci. Technol.* **2020**, *266*, 114533. DOI: 10.1016/j.anifeedsci.2020.114533.

Garcia-Caraballo, S.C.; Comhair, T.M.; Verheyen, F.; Gaemers, I.; Schaap, F.G.; Houten, S.M.; Hakvoort, T.B.; Dejong, C.H.; Lamers, W.H.; Koehler, S.E. Prevention and reversal of hepatic steatosis with a high-protein diet in mice. *Biochimica et Biophysica Acta -Molecular Basis of Disease* **2013**, *1832*, 685–695. DOI: 10.1016/j.bbadis.2013.02.003.

Yu, D.; Zhu, W.; Hang, S. Effects of low-protein diet on the intestinal morphology, digestive enzyme activity, blood urea nitrogen, and gut microbiota and metabolites in weaned pigs. *Arch. Anim. Nutr.* **2019**, *73*, 287–305. DOI: 10.1080/1745039X.2019.1614849.

Bong, H.Y.; Kim, J.Y.; Jeong, H.I.; Moon, M.S.; Kim, J.; Kwon, O. Effects of corn gluten hydrolyzates, branched chain amino acids, and leucine on body weight reduction in obese rats induced by a high fat diet. *Nutr Res Pract* **2010**, *4*, 106–113. DOI: 10.4162/nrp.2010.4.2.106.

Lin, X.; Li, L.; Gou, Z.; Fan, Q.; Wang, Y.; Jiang, S. Reproductive performance, metabolism and oxidative stress profile in Chinese yellow-feathered broiler breeder hens fed multiple levels of isoleucine. *British Poultry Science* **2021**, *62*, 509–516. DOI: 10.1080/00071668.2021.1894322.

Richert, B.; Goodband, R.D.; Tokach, M.D.; Nelssen, J.L. Increasing valine, isoleucine, and total branched-chain amino acids for lactating sows. *J. Anim. Sci.* **1997**, *75*, 2117–2128. DOI: 10.2527/1997.7582117x.

Kwon, W.B.; Touchette, K.J.; Simongiovanni, A.; Syriopoulos, K.; Wessels, A.; Stein, H.H. Excess dietary leucine in diets for growing pigs reduces growth performance, biological value of protein, protein retention, and serotonin synthesis. *J. Anim. Sci.* **2019**, *97*, 4282–4292. DOI: 10.1093/jas/skz259.

Emmanuel, B. Autoregulation of urea cycle by urea in mammalian species. *Comp. Biochem. Physiol.* **1981**, *70*, 79–81. DOI: 10.1016/0300-9629(81)90398-4.

VITA

Parniyan Goodarzi

Candidate for the Degree of

Doctor of Philosophy

Dissertation: DETERMINING THE ROLE OF TRYPTOPHAN, ISOLEUCINE AND VALINE IN REGULATION OF GROWTH AND NUTRIENT METABOLISM IN YOUNG PIGS

Major Field: Animal Science

Biographical:

Education:

Completed the requirements for the Doctor of Philosophy in Animal Science at Oklahoma State University, Stillwater, Oklahoma in December, 2022.

Completed the requirements for the Master of Science in Animal Physiology at Shiraz University, Shiraz, Fars/Iran in 2017.

Completed the requirements for the Bachelor of Science in Animal Science at Shiraz University, Shiraz, Fars/Iran in 2015.

Experience:

2019-2022 Graduate research and teaching assistant, Department of Animal and Food Sciences, Oklahoma State University.
2015-2022 Research experiences in several species including poultry, and swine
2021-2022 Peer review activities for several journals including Scientific Reports, Livestock Science, Frontiers and Tropical

Professional Memberships:

2019 – present: American Society of Animal Science (ASAS)
2019 – present: American Society for Nutrition (ASN)