Dietary glutamine, glutamate, and aspartate supplementation improves hepatic lipid metabolism in post-weaning piglets

Ming Qi a, b, 1, Jing Wang a, 1, Bi’e Tan a, *, Jianjun Li a, Simeng Liao a, b, Yanhong Liu c, Yulong Yin a

a Laboratory of Animal Nutritional Physiology and Metabolic Process, Key Laboratory of Agro-ecological Processes in Subtropical Region, National Engineering Laboratory for Pollution Control and Waste Utilization in Livestock and Poultry Production, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China
b University of Chinese Academy of Sciences, Beijing 100008, China
c Department of Animal Science, University of California, Davis 95616, CA, USA

ARTICLE INFO

Article history:
Received 11 September 2019
Received in revised form 3 December 2019
Accepted 4 December 2019
Available online 23 January 2020

Keywords:
Glutamate
Aspartate
Glutamine
Post-weaning piglets
Hepatic lipid metabolism

ABSTRACT

A previous study has demonstrated that early weaning significantly suppressed hepatic glucose metabolism in piglets. Glutamate (Glu), aspartate (Asp) and glutamine (Gln) are major metabolic fuels for the small intestine and can alleviate weaning stress, and therefore might improve hepatic energy metabolism. The objective of this study was to investigate the effects of administration of Glu, Asp and Gln on the expression of hepatic genes and proteins involved in lipid metabolism in post-weaning piglets. Thirty-six weaned piglets were assigned to the following treatments: control diet (Control; basal diet + 15.90 g/kg alanine); Asp, Gln and Glu-supplemented diet (Control + AA; basal diet + 1.00 g/kg Asp + 5.00 g/kg Glu + 10.00 g/kg Gln); and the energy-restricted diet supplemented with Asp, Gln and Glu (Energy / C0 + AA; energy deficient diet + 1.00 g/kg Asp + 5.00 g/kg Glu + 10.00 g/kg Gln). Liver samples were obtained on d 5 and 21 post-weaning. Piglets fed Energy / C0 + AA diet had higher liver mRNA abundances of acyl-CoA oxidase 1 (ACOX1), succinate dehydrogenase (SDH), mitochondrial transcription factor A (TFAM) and sirtuin 1 (SIRT1), as well as higher protein expression of serine/threonine protein kinase 11 (LKB1), phosphor-acetyl-CoA carboxylase (P-ACC) and SIRT1 compared with piglets fed control diet (P < 0.05) on d 5 post-weaning. Control + AA diet increased liver malic enzyme 1 (ME1) and SIRT1 mRNA levels, as well as protein expression of LKB1 and P-ACC on d 5 post-weaning (P < 0.05). On d 21 post-weaning, compared to control group, Glu, Gln and Asp supplementation up-regulated the mRNA levels of ACOX1, ME1 and SIRT1 (P < 0.05). These findings indicated that dietary Glu, Gln and Asp supplementation could improve hepatic lipid metabolism to some extent, which may provide nutritional intervention for the insufficient energy intake after weaning in piglets.

1. Introduction

Inadequate energy supply can cause metabolic disorders, which are involved in many health problems, such as lipodystrophy (Kang et al., 2015). Piglets are born with limited energy stores (Dividich et al., 2005), and limited capacity for oxidizing fatty acids and amino acids (Mersmann et al., 1984). Most deaths of piglets are caused by insufficient energy supply (Pettigrew, 1981). The liver plays a crucial role in whole-body energy utilization (McBride and Kelly, 1990), and maintaining the hepatic normal energy metabolism is important to ensure its normal function (Li et al., 2012).
Glutamate (Glu), glutamine (Gln) and aspartate (Asp) are members of the arginine family (Wu et al., 2007) and, traditionally, are classified as non-essential amino acids in mammals (Wu et al., 2013). They are interconvertible via complex metabolic pathways in most mammals, including pigs (Wu, 2009). Growing evidence shows that they play important roles in multiple signaling pathways, thereby regulating gene expression, nutrient metabolism, and energy requirements (Brasse-Laguel et al., 2009; Watford, 2008; Yao et al., 2008). Glutamine has been shown to induce enhanced intestinal secretory immunoglobulin A level which is important for mucosal defense (Ren et al., 2016). It also takes part in promoting macrophages from an M1 to an M2 phenotype, which may be prevention for disorder of lipid metabolism (Ren et al., 2019). During the process of absorption in the small intestine, metabolism of Glu, Gln and Asp, but not glucose, provides the main energy for the gut maintaining integrity and function (Watford, 2008; Windmueller and Spaeht, 1980). Glutamate can be the substrate for protein synthesis and metabolized to yield glucose via hepatic gluconeogenesis (Watford, 2008). Recent studies in infant pigs showed that when Glu is fed 4-fold higher than normal, most Glu molecules are either oxidized to supply energy or metabolized into other nonessential amino acids in the gut (Burrin and Stoll, 2008). Growing evidence shows that Asp is one of the major energy sources producing ATP, such as in mammalian enterocytes, through moderating tricarboxylic acid (TCA) cycle intermediates (Wu et al., 2013; Pi et al., 2014; Russell and Taegtmeyer, 1991; Rosenfeldt et al., 1998).

In piglets, studies to date have mainly focused on the role of these 3 amino acids as energy sources for intestinal cells and tissues (Ando, 1988; Blachier et al., 2009; Burrin and Stoll, 2009; Caballero-Solares et al., 2015), and their beneficial effects on the maintenance of the intestinal structure and function (Wu et al., 2011; Shan et al., 2012; Cabrera et al., 2013). A significant part of these 3 amino acids is transported to the liver (Brosnan, 2003), which is the major organ involved in nutrient assimilation and transformation into oxidizable substrates, such as glucose and fatty acids (Caballero-Solares et al., 2015). Weaning is associated with alteration of intestinal morphology and impaired ability of nutrition absorption (Ren et al., 2018). Previous study demonstrated that early weaning significantly suppressed glucose metabolism of the liver in piglets (Xie et al., 2016). To date, few detailed studies have reported the effects of amino acids on the hepatic energy metabolism of post-weaning piglets. The present study was conducted to investigate the effects of administration of Glu, Gln and Asp on the hepatic mRNA expression related to energy metabolism and the protein levels involved in adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) signaling pathway in the post-weaning piglets.

2. Materials and methods

2.1. Animals and experimental design

The animal experiments were approved by the Institutional Animal Care and Use Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences (2013020). Thirty-six healthy piglets (Duroc × Landrace × Large Yorkshire) weaned at 21 d of age were randomly assigned to 3 treatments (12 pigs/treatment) based on similar body weights, as follows: control diet (Control, basal diet + 15.90 g/kg alanine), Asp, Gln and Glu-supplemented diet (Control + AA, basal diet + 1.00 g/kg Asp + 5.00 g/kg Glu + 10.00 g/kg Gln) and the energy-restricted diet supplemented with Asp, Gln and Glu (Energy + AA, energy deficient diet + 1.00 g/kg Asp + 5.00 g/kg Glu + 10.00 g/kg Gln). The dose was based on the growth performance of piglets in the preliminary experiment. Alanine was used as an isonitrogenous control, as described by Yao et al., (2008). The piglets were housed individually in an environmentally controlled nursery with hard plastic slatted flooring, and fed ad libitum. All animals had free access to drinking water. The composition and nutrient levels of the diets met the nutrient requirements for weaning piglets according to recommendations of the NRC (2012) (Table 1). Eighteen weaned piglets (6 from each group) were slaughtered on d 5 and 21 post-weaning, respectively. There was no difference in body weight (6.89, 6.91, 6.94 kg, P = 0.952) on d 5 post-weaning and has a significant difference in body weight (11.58, 13.17, 12.07 kg, P = 0.047) on d 21 post-weaning. Liver samples were obtained, and then immediately snap-frozen in liquid nitrogen and stored at −80 °C for RNA extraction and Western blot analysis.

2.2. Real-time quantitative PCR (RT-qPCR)

The abundances of mRNA for acetyl-CoA carboxylase (ACC), acyl-CoA oxidase 1 (ACOX1), succinate dehydrogenase (SDH), adipose triglyceride lipase 4 (ATGL4) nuclear respiratory factor 1 (NRF1), mitochondrial transcription factor A (TFAM), malic enzyme 1 (ME1), peroxisome proliferator-activated receptor α (PPARα), phospho-enolpyruvate carboxykinase 1 (PCK1), sirtuin 1 (SIRT1), pyruvate

| Table 1 | Ingredients and nutrient composition of the diets (g/kg, as-fed basis). 1 |
|---------|-----------------------------|
| Item    | Control | Control + AA | Energy + AA |
| Corn    | 239.30 | 240.00 | 244.00 |
| Extruded corn | 350.00 | 350.00 | 350.00 |
| Soybean | 80.00 | 80.00 | 118.00 |
| Fermented soybean | 90.00 | 90.00 | 40.00 |
| Extruded soybean | 0.00 | 0.00 | 28.00 |
| Whey powder | 60.00 | 60.00 | 60.00 |
| Fish meal | 40.00 | 40.00 | 40.00 |
| Plasma protein powder | 20.00 | 20.00 | 20.00 |
| Soybean oil | 10.00 | 10.00 | 0.00 |
| Glucose | 30.00 | 30.00 | 30.00 |
| Sucrose | 20.00 | 20.00 | 0.00 |
| 98% L-lysine | 4.00 | 4.00 | 4.00 |
| DL-methionine | 1.10 | 1.10 | 1.10 |
| L-threonine | 1.20 | 1.20 | 1.20 |
| Alanine | 15.90 | 15.90 | 15.90 |
| Glutamine | 0.00 | 10.00 | 10.00 |
| Glutamate | 0.00 | 5.00 | 5.00 |
| Aspartate | 0.00 | 1.00 | 1.00 |
| Carrier | 9.00 | 8.20 | 48.20 |
| Organic acid calcium | 6.00 | 6.00 | 6.00 |
| Dicalcium phosphate | 10.00 | 10.00 | 10.00 |
| Choline chloride, 50% | 0.10 | 0.10 | 0.10 |
| Antioxidant | 0.05 | 0.50 | 0.50 |
| Mineral premix 2 | 1.50 | 1.50 | 1.50 |
| Vitamin premix 2 | 0.40 | 0.40 | 0.40 |
| ZnO | 4.00 | 4.00 | 4.00 |
| Acidifier | 7.00 | 7.00 | 7.00 |
| Total | 1,000.00 | 1,000.00 | 1,000.00 |

1. Control, basal diet + 15.90 g/kg alanine; Control + AA, basal diet + 1.00 g/kg aspartate + 5.00 g/kg glutamate + 10.00 g/kg glutamine; Energy + AA, energy deficient diet + 1.00 g/kg aspartate + 5.00 g/kg glutamate + 10.00 g/kg glutamine.

2. Mineral premix provided the following for 1 kilogram of diet: Zn (ZnO), 50 mg; Cu (CuSO4), 20 mg; Mn (MnO), 55 mg; Fe (FeSO4), 100 mg; I (KI), 1 mg; Co (CoSO4), 2 mg; Se (Na2SeO3), 0.3 mg. 

3. Vitamin premix provided the following for 1 kilogram of diet: vitamin A, 8,255 IU; vitamin D3, 2,000 IU; vitamin E; 40 IU; vitamin B1, 2 mg; vitamin B2, 4 mg; pantothenic acid, 15 mg; vitamin B6, 10 mg; vitamin B12, 0.05 mg; nicotinic acid, 30 mg; folic acid, 2 mg; vitamin K3, 1.5 mg; biotin, 0.2 mg; choline chloride, 800 mg; and vitamin C, 100 mg.
dehydrogenase kinase 4 (PDK4), uncoupling protein 2 (UCP2), alpha-ketoglutarate-dependent dioxygenase (FTO), carnitine palmitoyltransferase 1 (CPT1) in the liver were determined by RT-qPCR. Total RNA was isolated from the liquid nitrogen-pulverized liver samples with the TRIZOL reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. Synthesis of the first strand (cDNA) was performed with 5 × PrimeScript Buffer2 and PrimeScript reverse transcriptase Enzyme Mix 1 (TaKaRa Biotechnology [Dalian] Co., Ltd, Dalian, China). Primers were designed with Primer 5.0 (PREMIER Biosoft International, Palo Alto, CA, USA) according to the gene sequence of the pig to produce an amplification product, as described previously (Wang et al., 2015). The primers used to amplify genes are shown in Table 2. β-actin was used as a housekeeping gene to normalize target gene transcript levels (Tan et al., 2009). The reaction was performed in a volume of 10 μL (ABI Prism 7700 Sequence Detection System; Applied Biosystems, Foster City, CA, USA). The relative expression levels of the selected genes normalized against the reference gene (β-actin) were calculated by using the 2^(-△△Ct) method (Livak and Schmittgen, 2001), and data are expressed relative to those in control diet-treated piglets.

2.3. Western blot analysis

Liver samples were homogenized and protein concentrations were measured using the bicinchoninic acid (BCA) method (Beyotime Institute of Biotechnology, Beijing, China). The protein levels of ACC, AMPK, serine/threonine protein kinase 11 (LKB1), phosphor-ACC (P-ACC), phosphor-AMPK (P-AMPK), peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC1α), and SIRT1 in the liver were determined by Western blot analysis as described previously (Wang et al., 2015). The following antibodies were used for protein quantification: ACC (1:1,000; Cell Signaling Technology, Massachusetts, USA); AMPK (1:800; Santa Cruz Biotechnology, Texas, USA); LKB1 (1:1,000; LifeSpan Biosciences, Washington State, USA); P-ACC (1:1,000; Cell Signaling Technology, Massachusetts, USA); P-AMPK (1:1,000; Cell Signaling Technology, Massachusetts, USA); PGC1α (1:1,000; Abcam, Cambridge, UK); SIRT1 (1:1,000; Cell Signaling Technology, Massachusetts, USA); and β-actin (1:2,000; Cell Signaling Technology, Massachusetts, USA) along with the secondary antibody horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (1:5,000; ZSGB Biological Technology, Beijing, China). All protein measurements were normalized to β-actin, and data are expressed relative to the values in control piglets.

2.4. Statistical analysis

All statistical analyses were performed by one-way ANOVA using SPSS software 20.0 (SPSS Inc., Chicago, IL, USA). The differences among treatments were evaluated using Turkey’s test. Results are presented as means with standard error of mean (SEM). Probability (P) values < 0.05 were taken to indicate statistical significance.

3. Results

3.1. Relative mRNA expression of energy metabolic genes in the liver

On d 5 post-weaning, the results showed that hepatic ME1 and SIRT1 mRNA abundances in Control + AA treated piglets, and the mRNA abundances of ACCX1, SDH, TFAM and SIRT1 in Energy + AA treated piglets were significantly higher than those in control piglets (P < 0.05) (Table 3). On d 21 post-weaning, compared with the control treatment, Control + AA treatment significantly up-regulated hepatic ACCX1, PGC1α, ME1, PDK4, PPARα, PCK1, SIRT1, TFAM, PDK4 and FTO mRNA levels related to energy metabolism in the liver of piglets on d 5 post-weaning.

### Table 2

| Gene | GenBank Accession No. | Primer sequences (5’-3’) |
|------|-----------------------|-------------------------|
| β-actin | XM_003124280.3 | F: TGGGGGCGTCCAGAAACCTG<br>R: AAGGGGCTTGATCTCCTTCTGT |
| ACC | NM_001114269 | F: AGTGTGGCGGCACTCCCTGAT<br>R: TGTGGACAGCCTAGATCCTG |
| ACOX1 | NM_001101028.1 | F: AGTCTCATTCACCCACCACTG<br>R: AGGTAGACGACACCCGCA |
| SDH | NM_003125659.5 | F: CCGTGTGTCCTCTTCCTATGG<br>R: TGGCCCTAAACACAGCGACT |
| ATGL4 | NM_001098805.1 | F: GCGCGGACTTGACCGAGCTG<br>R: CCACGGCCTGCCAAGGAG |
| NRF1 | NM_013985626.1 | F: CATGGCGTACCACTACGACAG<br>R: ACATGCTCAGGGATGTCG |
| TFAM | NM_001130211.1 | F: CCAGCCTAGGTGGTTTCTCA<br>R: TGCCAGCTGCCCTTAAGG |
| ME1 | NM_001924333.4 | F: GGCTGCCTAAACACAGGAGA<br>R: TGAAGTGCAACACACATTCG |
| PPARα | NM_001445526.1 | F: CAGTGCCTACCTGAGAGAC<br>R: GCTTACGCTGGAGTACCG |
| PCK1 | NM_001123158.1 | F: TCTGTCCTGAGCGGTCTCA<br>R: GTGGAGAACTTCTCGAGG |
| SIRT1 | NM_001145750.1 | F: TACCAGACGAGCTTCTACAGG<br>R: TGGAGGCTGAGCAAGAGTC |
| PDK4 | NM_001159306.1 | F: CTACAGCACAACACATCTGGA<br>R: CATCTCTCCCTATACGTGG |
| UCP2 | NM_214280.1 | F: CTACACATACTGCTCCTCTGA<br>R: ATCTTCCTGCTACGACCT |
| FTO | NM_00112692.1 | F: AGCAAGTCCTTCAGAGGACC<br>R: CCACGCTCAAAACATCGG |
| CPT1 | NM_001129805.1 | F: GCTATTCCCTGATCTTCGTG<br>R: GCAGCCTGCCTCTTTGGGATA |

### Table 3

| Item | Control | Control + AA | Energy + AA | SEM | P-value |
|------|---------|--------------|-------------|-----|---------|
| ACC | 1.00 | 1.07 | 1.00 | 0.08 | 0.905 |
| ACCX1 | 1.00^a | 1.11^b | 1.88^a | 0.13 | 0.021 |
| SDH | 1.00^b | 1.27^b | 2.53^a | 0.22 | <0.001 |
| TFAM | 1.00 | 1.40 | 1.01 | 0.13 | 0.265 |
| NRF1 | 1.00 | 0.70 | 0.92 | 0.08 | 0.263 |
| SIRT1 | 1.00^a | 1.29^b | 1.60^b | 0.10 | 0.016 |
| PPARα | 1.00 | 1.62^a | 1.33^b | 0.11 | 0.047 |
| PCK1 | 1.00 | 1.40 | 1.12 | 0.11 | 0.314 |
| SIRT1 | 1.00 | 1.67^b | 1.65^a | 0.13 | 0.027 |
| PDK4 | 1.00 | 2.45 | 1.88 | 0.32 | 0.180 |
| FTO | 1.00 | 1.47 | 1.70 | 0.14 | 0.127 |
| CPT1 | 1.00 | 1.23 | 1.17 | 0.08 | 0.555 |

ACC = acetyl-CoA carboxylase; ACCX1 = acetyl-CoA oxidase 1; SDH = succinate dehydrogenase; ATGL4 = adipose triglyceride lipase 4; NRF1 = nuclear respiratory factor 1; TFAM = mitochondrial transcription factor A; ME1 = malic enzyme 1; PPARα = peroxisome proliferator-activated receptor α; PCK1 = phosphoenolpyruvate carboxykinase 1; SIRT1 = sirtuin 1; PDK4 = pyruvate dehydrogenase kinase 4; UCP2 = uncoupling protein 2; FTO = alpha-ketoglutarate-dependent dioxygenase; CPT1 = carnitine palmitoyltransferase 1.

1. Values within a row with different superscripts differ significantly (P < 0.05).
ME1, and SIRT1 mRNA levels, and Energy+/AA treatment significantly increased hepatic mRNA expression of SDH in the piglets (P < 0.05) (Table 4).

3.2. Protein expressions related to energy metabolic pathway in the liver

The relative protein abundances involved in AMPK signaling pathway were shown in Fig. 1 and Table 5. Compared with control diet, Asp, Glu and Gln supplementation significantly increased the relative protein levels of LKB1 and P-ACC in the basal diet, as well as LKB1, P-ACC and SIRT1 in the liver of piglets on d 5 post-weaning in the energy-restricted diet (P < 0.05).

4. Discussion

The liver plays an important role in maintaining normal energy metabolism in the body through the sophisticated process, such as lipid metabolism, glucose homeostasis and mitochondrial oxidation (Wu et al., 2013; Xie et al., 2016). Intestinal metabolism of Glu, Gln and Asp provides majority of ATP in most of cellular activities (Wu, 2010). For the most of cellular activities, ATP is the main energy source. These amino acids can regulate physiological functions via the activation of different signaling pathways and various transcription factors (Brasse-Lagnel et al., 2009). In our current study, we found that supplementation of Glu, Gln, and Asp improved gene and protein expression associated with hepatic lipid metabolism in the post-weaning piglets.

Tricarboxylic acid cycle, a bridge between glycolysis and fatty acid β-oxidation, is an important pathway for ATP production in mammals (Kang et al., 2015). Aspartate can moderate TCA cycle intermediates, such as oxaloacetate and malate, and Asp amino-transferase can catalyze the transfer of an α-amino group between Asp and Glu (Yudkoff et al., 1994). Glutamine is converted into Glu and ammonia, and then into α-ketoglutarate, followed by entry into TCA cycle (Duee et al., 2007). The SDH, found in the inner mitochondrial membrane of eukaryotes, is the only enzyme that participates in both the TCA cycle and the electron transport chain, for catalyzing the oxidation of succinate to fumarate to supply ATP (Oyedotun and Lemire, 2004). Enhancing the SDH enzyme activity can accelerate the TCA cycle, and then increase the ATP production (Liu and Lin, 2011). The present results showed that compared with control group, energy-restricted diets supplemented with Glu, Gln and Asp beneficially increased the SDH mRNA expression. Similarly, previous study reported that pretreatment with Asp maintained the activity of cardiac TCA cycle enzymes in rats (Svikumar et al., 2008). This may be due to the supplementation of these 3 amino acids leading to improved hepatic energy status (Pi et al., 2014). The mechanism may be that these 3 amino acids change the availability of transcription factors of the SDH gene (Wu et al., 2011). Triglyceride (TG) is the major energy storage form. In the liver, synthesized TG is either stored in cytoplasmic droplets or secreted as very low density lipoprotein particles, which are transferred from the liver to other tissues (Yamazaki et al., 2005; Owen et al., 1997). Acetyl-CoA carboxylase is the rate limiting enzyme in de novo fatty acid synthesis (Liu et al., 1994). A previous study has reported that diet supplemented with Glu promoted lipid synthesis by enhancing ACC1 mRNA level in fish liver (Caballero-Solares et al., 2015). Our study showed that these amino acids failed to increase hepatic ACC mRNA expression. This can be explained by different species or diet composition. Besides, ME, critical enzyme for lipid synthesis, is an important malate metabolizing enzyme which catalyzes the

| Table 4 | Effects of administration of glutamate, glutamine and aspartate on mRNA levels related to energy metabolism in the liver of piglets on d 21 post-weaning. 1 |
|---------|----------------------------------------------------------------------------------|
| Item    | Control | Control + AA | Energy + AA | Energy + AA SEM | P-value |
| ACC     | 1.00    | 1.14         | 1.27        | 0.07            | 0.257   |
| ACOX1   | 1.00b   | 1.70a        | 1.21ab      | 0.11            | 0.038   |
| SDH     | 1.00ab  | 0.93ab       | 1.35a       | 0.07            | 0.008   |
| ATGL4   | 1.00    | 1.23         | 1.20        | 0.09            | 0.815   |
| Nrf1    | 1.00    | 1.21         | 1.00        | 0.06            | 0.230   |
| TFM     | 1.00    | 1.26         | 1.10        | 0.06            | 0.296   |
| ME1     | 1.00b   | 1.34ab       | 0.96ab      | 0.07            | 0.013   |
| PPARα   | 1.00    | 1.66         | 1.47        | 0.16            | 0.203   |
| PCK1    | 1.00    | 0.71         | 0.79        | 0.06            | 0.096   |
| SIRT1   | 1.00b   | 1.36a        | 0.89b       | 0.08            | 0.026   |
| PDK4    | 1.00    | 0.83ab       | 0.63b       | 0.06            | 0.037   |
| UCP2    | 1.00    | 0.65         | 0.86        | 0.06            | 0.069   |
| FTO     | 1.00    | 0.89         | 0.78        | 0.05            | 0.230   |
| CPT1    | 1.00    | 1.12         | 1.14        | 0.12            | 0.903   |

ACC = acetyl-CoA carboxylase; ACOX1 = acyl-CoA oxidase 1; SDH = succinate dehydrogenase; ATGL4 = adipose triglyceride lipase 4; NRF1 = nuclear respiratory factor 1; TFM = mitochondrial transcription factor A; ME1 = malic enzyme 1; PPARα = peroxisome proliferator-activated receptor α; PCK1 = phosphoenolpyruvate carboxykinase 1; SIRT1 = sirtuin 1; PDK4 = pyruvate dehydrogenase kinase 4; UCP2 = uncoupling protein 2; FTO = alpha-ketoglutarate-dependent dioxygenase; CPT1 = carnitine palmitoyltransferase 1.

ACC = acetyl-CoA carboxylase; AMPK = adenosine 5’-monophosphate (AMP)-activated protein kinase; LKB1 = serine/threonine protein kinase 11; P-ACC = phosphor-ACC; P-AMPK = phosphor-AMPK; PGC1α = peroxisome proliferator-activated receptor gamma coactivator-1 alpha; SIRT1 = sirtuin 1. 1 Values within a row with different superscripts differ significantly (P < 0.05). 2 n = 6 per treatment group. Data are expressed as relative values to those of control treated piglets on d 21 post-weaning. Control, basal diets + 15.90 g/kg alanine; Control + AA, basal diets + 1.00 g/kg aspartate + 5.00 g/kg glutamate + 10.00 g/kg glutamine; Energy+ AA, energy deficient diets + 1.00 g/kg aspartate + 5.00 g/kg glutamate + 10.00 g/kg glutamine.

Fig. 1. Representative Western blot images of serine/threonine protein kinase 11 (LKB1), phosphor-acetyl-CoA carboxylase (P-ACC), sirtuin 1 (SIRT1) and β-actin in the liver of piglets on d 5 post-weaning. Control, basal diets + 15.90 g/kg alanine; Control + AA, basal diets + 1.00 g/kg aspartate + 5.00 g/kg glutamate + 10.00 g/kg glutamine; Energy + AA, energy deficient diets + 1.00 g/kg aspartate + 5.00 g/kg glutamate + 10.00 g/kg glutamine. n = 6.
reversible oxidative decarboxylation of L-malate coupled with the reduction of dinucleotide cofactor nicotinamide adenine dinucleotide phosphate (NADP) and yields pyruvate and CO₂ (Yu and Ginsberg, 2004; Chang and Tong, 2003). Supplementation of Glu, Gln and Asp could increase the ME mRNA expression on d 5 and 21 post-weaning in the present study, respectively, which may be due to the enhancement in the ability of substrates binding at the active site of ME (Chang and Tong, 2003). In animals, fat deposition depends on the relative rate of TG synthesis and storage and of lipid mobilization and fatty acid oxidation (Reiter et al., 2007). Fatty acid β-oxidation is the vital pathway to lipid oxidation among which ACOX, CPT1 and UCP2 are critical enzymes (You et al., 2002). Acyl-CoA oxidase 1, the target gene of PPARα, the key factor in lipid metabolism, could promote lipid catabolism via regulating the process of fatty acid β-oxidation in mitochondria and peroxisome (Marcus et al., 1993; Martin et al., 1997). In the current study, ACOX1 mRNA expression was significantly increased in response to supplementation of Glu, Gln and Asp, confirming the roles of ACOX1 in the hepatic lipid catabolism function. Based on these results, these 3 amino acids may regulate the transcription of ACOX1 through alteration of the specificity of RNA polymerase for promoters (Wu et al., 2011). Glutamate, Gln and Asp may improve lipid catabolism by enhancing ACOX1 activity, increasing malonyl-CoA concentration and inhibiting CPT1 activity during lipogenesis (Scott et al., 1981).

Adenosine 5’-monophosphate (AMP)-activated protein kinase plays a key role as a master regulator of cellular energy homeostasis (Lee et al., 2011). There were no effects of Glu, Gln and Asp supplementation on P-AMPK and PGC1α protein expression, which may be due to that the hepatic energy changing fails to reach the range of AMPK phosphorylation perception (Wijesekara et al., 2018). However, Asp, Glu and Gln supplementation significantly increased the relative protein levels of LKB1 and P-ACC in the liver of piglets on d 5 post-weaning. Serine/threonine protein kinase 11 is the key upstream activator of the AMPK, and they can together control glucose and lipid metabolism in response to alterations in nutrients and intracellular energy levels (Shackelford and Shaw, 2009). Adenosine 5’-monophosphate (AMP)-activated protein kinase-ACC pathway contributes to fatty acid synthesis and oxidation. The increased amounts of AMPK induce a higher level of ACC phosphorylation, which results in decreased fatty acids synthesis (Kim et al., 2012). Addition of Asp, Glu and Gln to the energy-restricted diet also increased SIRT1 protein level in the current study, which agrees with the previous study that Asp supplementation increased mRNA expression of hepatic SIRT1 in the weaning pigs (Kang et al., 2015). Sirtuin 1 plays a role in inducing the mRNA expression of fatty acid oxidation genes (Lagouge et al., 2006), and it can also be activated by LKB1 (Shackelford and Shaw, 2009). Furthermore, SIRT1 was shown to de-acetylatelyte and affect the activity of PGC1α, culminating in the transcriptional regulation of mitochondrial and lipid metabolism genes (Pi et al., 2014). Sirtuin 1 also plays an important role in mediating inflammatory pathway (Xia et al., 2019). Based on the results, Glu, Gln and Asp may play an intermediary role in the interaction between LKB1 and SIRT1 (Peng et al., 2010). Microbiota and their metabolites have critical importance in intestinal immunity and lipid profiles (Ren et al., 2016; Yin et al., 2018), further study may focus on the interaction between gut microbiota and hepatic lipid metabolism.

5. Conclusions

The present results showed that dietary Glu, Gln and Asp supplementation increased hepatic gene expression involved in lipid metabolism and protein levels related to the AMPK signaling pathway in post-weaning piglets. These findings indicated that dietary Glu, Gln and Asp supplementation could improve hepatic lipid metabolism to some extent, which may provide nutritional intervention for the insufficient energy intake after weaning in piglets.

Conflict of Interest

We declare that there have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgements

This research was funded by Key Programs of frontier scientific research of the Chinese Academy of Sciences (QYZDY-SSW-SMC008), National Natural Science Foundation of China (No. 31672433, 31501964, 31560040), the Earmarked Fund for China Agriculture Research System (CARS-35) and Youth Innovation Team Project of ISA, CAS (2017QNCXTD_TBE).

References

Ando M. Amino-acid metabolism and water transport across the seawater Eel intestine. J Exp Biol 1968;138:93–106.
Blachier F, Bosry C, Bos C, Tome D. Metabolism and functions of L-glutamate in the epithelial cells of the small and large intestines. Am J Clin Nutr 2009;90(3):814S–215.
Brasse-Lagael C, Lavonnie A, Husson A. Control of mammalian gene expression by amino acids, especially glutamine. FEBS J 2009;276(7):1826–44.
Brosnan JT. Interorgan amino acid transport and its regulation. J Nutr 2003;133(6 Suppl 1):208S–725.
Burrin DG, Stoll B. Metabolic fate and function of dietary glutamate in the gut. Am J Clin Nutr 2009;90(3):850S–65.
Caballero-Solares A, Viegas I, Salgado MC, Siles AM, Sánchez F, Tomé I, Baanante IV, Fernández F. Diets supplemented with glutamate or glutamine improve protein retention and modulate gene expression of key enzymes of hepatic metabolism in gilthead seabream (Sparus aurata) juveniles. Aquaculture 2015;444:79–87.
Cabrera RA, Usry JL, Arrellano C, Nogueira ET, Moeser AJ, Odle J. Effects of creep feeding and supplemental glutamine or glutamine plus glutamate (Gln/Asp) on pre- and post-weaning growth performance and intestinal health of piglets. J Anim Sci Biotechnol 2013;4.
Chang GG, Tong L. Structure and function of malic enzymes, a new class of oxidative decarboxylases. Biochemistry 2003;42(44):12721–33.
Dovidich JL, Rooke JA, Herpin P. Nutritional and immunological importance of colostrum for the newborn pig. J Agric Sci 2005;143(6).
Dure´ P-H, Darcy-Villon B, Blachier F, Morel M-T. Fuel selection in intestinal cells. Proc Nutr Soc 2007;54(1):83–94.
Kang P, Liu Y, Zhu H, Li S, Shi H, Chen F, Leng W, Pi D, Hou Y, Yi D. The effect of aspartate on the energy metabolism in the liver of weaning pigs challenged with lipopolysaccharide. Eur J Nutr 2015;54(4):581–8.
Kim MK, Kim SH, Yu HS, Park HG, Kang AG, Ahn YM, Kim YS. The effect of clozapine on the AMPK-ACC-CPT1 pathway in the rat frontal cortex. Int J Neuro- psychopharmacol 2012;15(7):907–17.
Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Blachier F, Boutry C, Bos C, Tome D. Metabolism and functions of L-glutamate in the epithelial cells of the small and large intestines. Am J Clin Nutr 2009;90(3):814S–215.
Lee CY, Grant AL, Kim KH, Mills SE. Porcine somatotropin decreases acetyl-CoA carboxylase gene expression in porcine adipose tissue. Domest Anim Endocrinol 1994;11(1):125–32.
Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(T) (Delta Delta C) method. Methods 2001;25(4):402–8.
Marcus SL, Miyata KS, Zhang B, Subramani S, Rachubinski RA, Capone JP. Diverse peroxisome proliferator-activated receptors bind to the peroxisome proliferator-responsive elements of the rat hydratase/dehydrogenase and fatty
acetyl-CoA oxidase genes but differentially induce expression. Proc Natl Acad Sci U S A 1993;90(12):5723–7.

Martin G, Schoonjans K, LeFebvre AM, Staels B, Auwerx J. Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPARalpha and PPARgamma activators. J Biol Chem 1997;272(45):28210–7.

McBride BW, Kelly JM. Energy cost of absorption and metabolism in the ruminant gastrointestinal tract and liver: a review. J Anim Sci 1990;68(9).

Mersmann HJ, Pond WG, Yen JT. Use of carbohydrate and fat as energy source by obese and lean swine. J Anim Sci 1984;58(4):894.

NR Council. Nutrient Requirements of Swine: Eleventh Revised Edition. Washington, DC: The National Academies Press; 2012. 420 p.

Owen MR, Corstorphine CC, Zammit VA. Overt and latent activities of diacylglycerol acyltransferase in rat liver microsomes: possible roles in very-low-density lipoprotein triacylglycerol secretion. Biochem J 1997;323(Pt 1):17–21.

Oyedotun KS, Lemire BD. The quaternary structure of the Saccharomyces cerevisiae sucbinate dehydrogenase. Homology modeling, cofactor docking, and molecular dynamics simulation studies. J Biol Chem 2004;279(10):9424–31.

Peng YH, Rideout DA, Rakita SS, Gower WR, You M, Murr MM. Does LKB1 mediate mTORC1 activation of hepatic AMP-protein kinase (AMPK) and Sirtuin1 (SIRT1) after Roux-en-Y gastric bypass in obese rats? J Gastrointest Surg 2010;14(2):221–8.

Pettigrew JE. Supplemental dietary fat for peripartal sows: a review. J Anim Sci 1981;53(1).

Pla D, Liu Y, Shi H, Li S, Odle J, Lin X, Zhu H, Chen F, Hou Y, Leng W. Dietary supplementation of aspartate enhances intestinal integrity and energy status in weaning pigs after lipopolysaccharide challenge. J Nutr Biochem 2014;25(4):456–62.

Reiter RJ, Zhang D, Zhu C, Zhu G, Ren W, Yin Y. Melatonin reprogramming of gut microbiota improves lipid dysmetabolism in high-fat diet-fed mice. J Pineal Res 2019;66(2):e12547.

Xie C, Wang Q, Wang J, Tan B, Fan Z, Deng ZY, 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(4):824–30.

Yamazaki T, Sasaki E, Kakinuma C, Yano T, Miura S, Ezaki O. Increased very low density lipoprotein secretion and very low density lipoprotein receptor expression of the fatty acid transport protein and acyl-CoA synthetase genes by dietary fructooligosaccharides in ruminant gastrointestinal tract and liver: a review. J Anim Sci 2011;89(7):2:88–90.

You M, Fischer M, Deeg MA, Crabb DW. Ethanol induces fatty acid synthesis in the jejunal mucosa of neonatal pigs. J Nutr 2008;138(5):867–73.

Yu YH, Ginsberg HN. The role of acyl-CoA:diacylglycerol acyltransferase (DGAT) in energy metabolism. Adv Nutr 2019;10(2):321–31.

Yoon J, Lee H, Park J, Park JH, Hong JH. Glutamine metabolism and function in relation to proline synthesis and the safety of glutamine and proline supplementation. J Nutr 2008;138(10):2035–75.

Wijesekara N, Tung A, Thong F, Klip A. Muscle cell depolarization induces a gain in surface GLUT4 via reduced endocytosis independently of AMPK. Am J Physiol-Endoc M 2006;290(6):E1276–86.

Windmueller HG, Spaeth AE. Respiratory fuels and nitrogen metabolism in vivo in small intestine of fed rats. Quantitative importance of glutamine, glutamate, and aspartate. J Biol Chem 1980;255(1):107–12.

Wu G. Amino acids: metabolism, functions, and nutrition. Amino Acids 2009;37(1):1–17.

Wu G. Functional amino acids in growth, reproduction, and health. Adv Nutr 2010;1(1):31–7.

Wu G, Bazer FW, Davis TA, Jaeger LA, Johnson GA, Kim SW, Knabe DA, Meininger CJ, Spencer TE, Yin Y-L. Important roles for the arginine family of amino acids in swine nutrition and production. Livest Sci 2007;112(1–2):8–22.

Wu G, Bazer FW, Johnson GA, Knabe DA, Burghardt RC, Spencer TE, Li XL, Wang JJ. Triennial Growth Symposium: important roles for L-glutamine in swine nutrition and production. J Anim Sci 2011;89(7):2017–30.

Wu G, Wu Z, Dai Z, Yang Y, Wang W, Liu C, Wang B, Wang J, Yin Y. Dietary requirements of “nutritionally non-essential amino acids” by animals and humans. Amino Acids 2013;44(4):1107–13.

Xia Y, Chen S, Zeng S, Zhao Y, Zhu C, Deng B, Zhu G, Yin Y, Wang W, Hardeland R, Ren W. Melatonin in macrophage biology: current understanding and future perspectives. J Pineal Res 2019;66(2):e12547.

Xu C, Wang Q, Wang J, Tan B, Fan Z, Deng ZY, Wu X, Yin Y. Developmental changes in hepatic glucose metabolism in a newborn piglet model: a comparative analysis for sucking period and early weaning period. Biochem Biophys Res Commun 2016;470(4):824–30.

Yu YH, Ginsberg HN. The role of acyl-CoA:diacylglycerol acyltransferase (DGAT) in energy metabolism. Ann Med 2004;36(4):252–61.

Yudkoff M, Nelson D, Daikhin Y, Erecinska M. Tricarboxylic acid cycle in rat brain synaptosomes. Fluxes and interactions with aspartate aminotransferase and malate/aspartate shuttle. J Biol Chem 1994;269(44):27414–20.