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Original Research Article

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

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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⁻ + 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⁻ + 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.

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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).

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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-Lagnel 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 Spaeth, 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, 2009). 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 Taegtmeier, 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 (PPAR α), phosphoenolpyruvate 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
Ingredients			
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	0.00
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	0.00	0.00
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	0.50	0.50	0.50
Mineral premix ²	1.50	1.50	1.50
Vitamin premix ³	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
Calculated chemical composition			
Digestible energy, MJ/kg	14.44	14.40	13.52
Analyzed crude protein	195.605	195.34	195.29
Apparent digestible lysine	11.40	11.40	11.10

¹ 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.

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 μ L (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 $2^{-\Delta\Delta C_t}$ 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, Shanghai, 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 (*PGC1 α*), 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); *PGC1 α* (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 *ACOX1*, *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 *ACOX1*,

Table 2
Primers used for Real-time quantitative PCR.

Gene	GenBank Accession No.	Primer sequences (5'-3')
β -actin	XM_003124280.3	F: CTGCGGCATCCACGAAACT R: AGGGCCGTGATCTCCTTCTG
ACC	NM_001114269	F: ATGTTTCGGCAGTCCCTGAT R: TGTGGACCAGCTGACCTTGA
<i>ACOX1</i>	NM_001101028.1	F: TGTCAACTTCACCCCAACCTG R: AGGTAGGACACCATGCCACA
<i>SDH</i>	XM_003125659.5	F: CCGCTGGTCTTTCCTATGG R: TGGCCCTAAACACAGGGACT
<i>ATGL4</i>	NM_001098605.1	F: GCCACGAGTGATAGCATCC R: CAGCAGTTGGACAGGGTG
<i>NRF1</i>	XM_013985626.1	F: CATGGCACTCAACAGCGAAG R: ACATGCTCACAGGGATCTGG
<i>TFAM</i>	NM_001130211.1	F: CCACCTGAGTGGTTTTCCA R: TGCCAGTCTGCCCTATAAAG
<i>ME1</i>	XM_001924333.4	F: GGCTGCCTTAACACACGAGAA R: TGAATAATGCACCAATCCG
<i>PPARα</i>	NM_001044526.1	F: CAGTGCTACCTGACCGAGAC R: GCTTGACTGGGATGACCGAA
<i>PCK1</i>	NM_001123158.1	F: TCTGGTGTACGAGGCTCTCA R: TTGCCAAGTTGTAGCCGAA
<i>SIRT1</i>	NM_001145750.1	F: TACCAGAGCAGTTTCATAGAGCC R: TGCAGGTGAGGCAAAGGTTT
<i>PDK4</i>	NM_001159306.1	F: CTACAGCACCAACACCTGTGA R: CATCCGTTCCATATCTGGCA
<i>UCP2</i>	NM_214289.1	F: CTCACCAATGTCGCTCGTA R: ATCTCGTCTTGACCACGTCC
<i>FTO</i>	NM_001112692.1	F: AGAATGTCCTGATGAGGCG R: CAGCCACTCAAACCTCGACCT
<i>CPT1</i>	NM_001129805.1	F: GCATTTGCCATCTTTCGT R: GCACTGGTCTTCTGGGATA

ACC = acetyl-CoA carboxylase; *ACOX1* = acyl-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; *PPAR α* = peroxisome proliferator-activated receptor α ; *PCK1* = phosphoenolpyruvate carboxylase 1; *SIRT1* = sirtuin 1; *PDK4* = pyruvate dehydrogenase kinase 4; *UCP2* = uncoupling protein 2; *FTO* = alpha-ketoglutarate-dependent dioxygenase; *CPT1* = carnitine palmitoyltransferase 1.

Table 3

Effects of administration of glutamate, glutamine and aspartate on mRNA levels related to energy metabolism in the liver of piglets on d 5 post-weaning.¹

Item	Control	Control + AA	Energy ⁻ + AA	SEM	<i>P</i> -value
ACC	1.00	1.07	1.00	0.08	0.905
<i>ACOX1</i>	1.00 ^b	1.11 ^b	1.88 ^a	0.13	0.021
<i>SDH</i>	1.00 ^b	1.27 ^b	2.53 ^a	0.22	<0.001
<i>ATGL4</i>	1.00	1.40	1.01	0.13	0.265
<i>NRF1</i>	1.00	0.70	0.92	0.08	0.263
<i>TFAM</i>	1.00 ^b	1.29 ^{ab}	1.66 ^a	0.10	0.016
<i>ME1</i>	1.00 ^b	1.62 ^a	1.33 ^{ab}	0.11	0.047
<i>PPARα</i>	1.00	1.40	1.12	0.11	0.314
<i>PCK1</i>	1.00	1.40	1.35	0.13	0.385
<i>SIRT1</i>	1.00 ^b	1.67 ^a	1.69 ^a	0.13	0.027
<i>PDK4</i>	1.00	2.45	1.88	0.32	0.180
<i>UCP2</i>	1.00	1.47	1.70	0.14	0.127
<i>FTO</i>	1.00	1.23	1.12	0.08	0.555
<i>CPT1</i>	1.00	1.26	1.17	0.12	0.667

ACC = acetyl-CoA carboxylase; *ACOX1* = acyl-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; *PPAR α* = peroxisome proliferator-activated receptor α ; *PCK1* = phosphoenolpyruvate carboxylase 1; *SIRT1* = sirtuin 1; *PDK4* = pyruvate dehydrogenase kinase 4; *UCP2* = uncoupling protein 2; *FTO* = alpha-ketoglutarate-dependent dioxygenase; *CPT1* = carnitine palmitoyltransferase 1.

^{a, b}Values within a row with different superscripts differ significantly (*P* < 0.05).

¹ *n* = 6 per treatment group. Data are expressed as relative values to those of control treated 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.

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 aminotransferase 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 (Duée 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 (Sivakumar 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	SEM	P-value
ACC	1.00	1.14	1.27	0.07	0.257
ACOX1	1.00 ^b	1.70 ^a	1.21 ^{ab}	0.11	0.038
SDH	1.00 ^b	0.93 ^b	1.35 ^a	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
TFAM	1.00	1.26	1.10	0.06	0.296
ME1	1.00 ^b	1.34 ^a	0.96 ^b	0.07	0.013
PPAR α	1.00	1.66	1.47	0.16	0.203
PCK1	1.00	0.71	0.79	0.06	0.096
SIRT1	1.00 ^b	1.36 ^a	0.89 ^b	0.08	0.026
PDK4	1.00 ^a	0.83 ^{ab}	0.63 ^b	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; TFAM = mitochondrial transcription factor A; ME1 = malic enzyme 1; PPAR α = 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.

^{a, b}Values within a row with different superscripts differ significantly ($P < 0.05$).

¹ $n = 6$ 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.

Table 5

Effects of administration of glutamate, glutamine and aspartate on protein expression related to energy metabolism in the liver of piglets on d 5 post-weaning.¹

Item	Control	Control + AA	Energy ⁻ +AA	SEM	P-value
ACC	1.00	0.72	1.90	0.28	0.209
AMPK	1.00	0.65	1.56	0.26	0.408
LKB1	1.00 ^b	3.29 ^a	3.84 ^a	0.49	0.005
P-ACC	1.00 ^b	2.24 ^a	1.86 ^a	0.18	0.012
P-AMPK	1.00	1.07	0.81	0.14	0.788
PGC1 α	1.00	0.81	1.65	0.25	0.385
SIRT1	1.00 ^b	2.87 ^b	3.86 ^a	0.45	0.031

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; PGC1 α = peroxisome proliferator-activated receptor gamma coactivator-1 alpha; SIRT1 = sirtuin 1.

^{a, b}Values within a row with different superscripts differ significantly ($P < 0.05$).

¹ $n = 6$ 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.

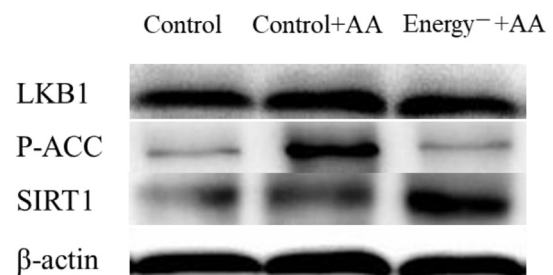


Fig. 1. Representative Western blot images of serine/threonine protein kinase 11 (LKB1), phosphor-acetyl-CoA carboxylase (P-ACC), sirtuin 1 (SIRT1) and β -actin in the liver of piglets on d 5 post-weaning. Control, basal diets + 15.90 g/kg alanine; Control + AA, basal diets + 1.00 g/kg aspartate + 5.00 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 *PPAR α* , 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 PGC1 α protein expression, which may be due to that the hepatic energy changing fails to reach the range of AMPK phosphorylation perception (Wijesekara et al., 2006). 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 weanling 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-acetylate and affect the activity of PGC1 α , 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 we 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.

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