Pyruvate is an effective substitute for glutamate in regulating porcine nitrogen excretion

Yunxia Li,^{*,†} Zhiru Tang,^{†,1} Tiejun Li,^{‡,1} C. Chen,[†] Feiruo Huang,^{||} Jing Yang,[†] Qingqing Xu,[†] Jifu Zhen,[†] Zhaoliang Wu,[†] Mao Li,[†] Jiajing Sun,[†] Jinchao Chen,[†] Xiangxin Zhang,[†] Liuting Wu,[†] Rui An,[†] Shengjun Zhao,[§] Qingyan Jiang,[¶] Weiyun Zhu,^{**} Yulong Yin,[‡] and Zhihong Sun^{†,2}

^{*}Institute of Animal Nutrition, Sichuan Agricultural University, Chengdu 611130, P. R. China; [†]Laboratory for Bio-feed and Molecular Nutrition, College of Animal Science and Technology, Southwest University, Chongqing 400715, P. R. China; [‡]Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha 410125, P. R. China; ^{||}College of Animal Science and Technology, Huazhong Agricultural University, Wuhan 430070, P. R. China; [§]School of Animal Science and Nutritional Engineering, Wuhan Polytechnic University, Wuhan 430023, P. R. China; [¶]College of Animal Science and Technology, Huanan Agricultural University, Guangzhou 510642, P. R. China; and ^{**}College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, P. R. China

ABSTRACT: This study was performed to determine if pyruvate, which acts as a critical intermediate in energy metabolism, can substitute the role of glutamate as a metabolic fuel and effectively reduce nitrogen excretion in pigs. First, the experiment in vitro was carried out to investigate the effects of culturing porcine small intestinal epithelial cell line with pyruvate on the oxidation. Then, barrows weighing 40 kg were used in the experiment investigating the changes of nitrogen balance in response to addition of pyruvate to low-protein diets. Last, barrows (40 kg), which were surgically fitted with permanent catheters in the mesenteric vein, portal vein, hepatic vein, and carotid artery, were used to investigate the effects of supplementing low-protein diets with calcium pyruvate on the net portal fluxes of amino acids (AAs) and the consumption of AAs in the liver. The results showed that culturing cells

with sodium pyruvate significantly reduced the number of glutamate oxidation ($P < 0.05$). Addition of calcium pyruvate to low-protein diets significantly reduced urinary nitrogen excretion from 13.2 g/d (18.0% crude protein, CP) to 10.3 g/d (15.0% CP) or 7.80 g/d (13.5% CP) and total nitrogen excretion from 22.5 g/d (18.0% CP) to 17.8 g/d (15.0% CP) or 14.2 g/d (13.5% CP) ($P < 0.05$), without obviously negative effects on the nitrogen retention ($P > 0.05$). Addition of calcium pyruvate to low-protein diets significantly decreased essential AA consumption rate in the liver ($P < 0.05$). This diet modification reduced the net portal fluxes of NH_3 , glycine, and alanine, as well as urea production rate in the liver ($P < 0.05$). The results indicated that pyruvate is an effective substitute for glutamate as a supplement in low-protein diets, reducing porcine nitrogen excretion and nitrogen consumption.

Key words: amino acid metabolism, low-protein diet, nitrogen excretion, pig, pyruvate

© The Author(s) 2018. Published by Oxford University Press on behalf of the American Society of Animal Science. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com.

J. Anim. Sci. 2018.96:3804–3814

doi: 10.1093/jas/sky237

¹Equally to first author.

²Corresponding author: sunzh2002cn@aliyun.com

Received December 10, 2017.

Accepted June 10, 2018.

INTRODUCTION

Reducing dietary crude protein (CP) intake is an effective way to decrease nitrogen excretion in growing-finishing pigs (Tuitoek et al., 1997;

Le Bellego et al., 2002; Kerr et al., 2003; Shriver et al., 2003; Hinson et al., 2009; Galassi et al., 2010; Hernández et al., 2011; Gallo et al., 2014; Luo et al., 2015; Hong et al., 2016; Zhang et al., 2016; Li et al., 2017) and address nitrogen pollution from the livestock industry. However, this benefit typically comes at the expense of poor growth performance when dietary CP content is reduced by $\geq 3\%$ (Kerr and Easter, 1995; Figueroa et al., 2002; Otto et al., 2003; He et al., 2016). Thus, there is considerable interest in understanding exactly how low-protein diets limit growth in pigs. In order to address this problem, we carried out a series of prior studies and came to the following conclusions (Chen, 2015; Wu, 2016; Yang, 2016; Xu, 2017).

First, we showed that efforts to reduce nitrogen excretion should focus on urinary nitrogen and inhibiting the entrance of NH_3 , glycine, and alanine to the liver is an important strategy for reducing urinary nitrogen. Our previous works found that diet-induced changes to amino acid (AA) metabolism (decreasing the supply of non-essential AA [NEAA] to the parenteral tissues and increasing the consumption of essential AA [EAA] in the liver) are a major cause of the growth-limiting effects. We also demonstrated that glutamate addition mitigates this disadvantage through balancing the AA profile from the portal-drained viscera (PDV), consequently minimizing EAA consumption in the liver. However, glutamate supplementation of low-protein diets increased dietary nitrogen consumption, resulting in the need for a substitute that could reduce nitrogen excretion without also limiting growth.

The main purpose of glutamate addition is to increase metabolic fuel to the gut, as glutamate is the primary oxidative substrate for intestinal mucosa (Stoll et al., 1998; Reeds et al., 2000; Newsholme et al., 2003). Pyruvate is a major metabolic intermediate of glucose and is involved in numerous pathways, including cytoplasmic glycolysis, mitochondrial carbohydrate oxidation, glucose production, fatty acid biosynthesis, and AA metabolism (Bricker et al., 2012). Pyruvate is critical to energy metabolism (Bricker et al., 2012; Vacanti et al., 2014; Gray et al., 2015) but does not generate nitrogen sources for urea production. Previous findings also demonstrated that AA metabolism is deeply influenced by pyruvate flux into mitochondrial pathways (Vacanti et al., 2014; Gray et al., 2015). Therefore, we hypothesized that pyruvate could replace glutamate as a metabolic fuel, reducing nitrogen excretion without negatively affecting porcine growth performance.

MATERIALS AND METHODS

Animal Use and Care

All experimental procedures were in accordance with the Animal Care and Use Guidelines of Southwest University, Chongqing, China.

Crossbred (Yorkshire-Landrace sow $\times$ Duroc boar) pigs, which obtained from a local commercial swine farm, were individually placed in stainless-steel metabolic cages. The basal diets (Table 1) were formulated according to National Research Council recommendations and 4 EAA (lysine, threonine, methionine, and tryptophan) were added to the diets to meet AA standards (NRC, 2012). All pigs had ad libitum access to fresh water. The amount of feed supplied to the pigs daily in the low-protein diet group was the same as that in the control group. Room temperature was maintained at approximately 26 °C (range, 25.4 to 26.6 °C) with thermostatically controlled heaters and exhaust fans.

Cellular Oxidative Metabolism

Porcine small intestinal epithelial cell line (IPEC-J2) were seeded in 25-cm² culture flasks with DMEM media (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS) (Thermo Fisher Scientific Inc., Grand Island, NY, USA) and 1% penicillin/streptomycin (Hyclone Laboratories, Logan, UT, USA) including 110 or 400 mg/liter sodium pyruvate (Sigma-Aldrich, St. Louis, MO, USA). Simultaneously, [$U\text{-}^{13}\text{C}_3$]-pyruvate or [$U\text{-}^{13}\text{C}_5$]-Glu (0.1 $\mu\text{Ci}/\text{mL}$; Sigma-Aldrich) was added to culture medium. The methods for determining $^{13}\text{CO}_2$ production were referred to the procedures described previously (Abe et al., 2006). Briefly, the flasks were capped with rubber stoppers containing a center well and incubated at 37 °C for 60 min. The $^{14}\text{CO}_2$ produced during incubation was trapped by hyamine hydroxide. The reaction was blocked with 250 μL of 60% perchloric acid, and the flasks were kept at 4 °C overnight to trap the $^{14}\text{CO}_2$. The center wells were transferred to 20-mL glass scintillation counter vials with 550 μL of ethanol and 10 mL of scintillator liquid. The ^{14}C contents of the vials were assayed on a liquid scintillation counter. The results were normalized based on cellular protein levels assayed by Bradford assay using BSA as a standard.

Nitrogen Balance

In one our previous study, we already investigated the effects of supplementing low-protein

Table 1. Ingredients and composition of diets differing in CP content, fed to 30- to 60-kg pigs (dry matter basis)

Ingredients (%)	Diets		
	18.0% CP	15.0% CP	13.5% CP
Corn	62.35	70.59	75.30
Soybean meal	25.20	16.20	11.10
Wheat bran	7.80	6.88	5.97
Soybean oil	1.56	2.22	2.40
Lysine	0.21	0.42	0.55
Methionine	0.01	0.08	0.16
Threonine	0.01	0.13	0.25
Tryptophan	0.00	0.03	0.08
Dicalcium phosphate	0.67	0.76	1.02
Calcium carbonate	0.89	0.59	0.07
Salt	0.30	0.30	0.30
Glucose	0.00	0.80	1.80
1% premix ¹	1.00	1.00	1.00
Total	100.00	100.00	100.00
Composition (% unless indicated otherwise)			
ME (MJ/kg) ²	13.9	13.9	13.9
CP ³	18.0	15.0	13.6
SID Lys ²	0.96	0.96	0.97
SID Met + Cys ²	0.56	0.56	0.55
SID Thr ²	0.62	0.62	0.61
SID Trp ²	0.17	0.17	0.17
SID Arg ²	1.04	0.90	0.81
SID His ²	0.40	0.35	0.30
SID Ile ²	0.62	0.54	0.48
SID Leu ²	1.31	1.14	1.01
SID Phe ²	0.75	0.64	0.58
SID Val ²	0.61	0.55	0.49
Ca ³	0.62	0.62	0.61
P ³	0.51	0.50	0.50

¹Composition per kg of premix: 119 g MgSO₄·H₂O, 2.5 g FeSO₄·7H₂O, 0.8 g CuSO₄·5H₂O, 3 g MnSO₄·H₂O, 5 g ZnSO₄·H₂O, 10 mg Na₂SeO₃, 40 mg KI, 30 mg CoCl₂·6H₂O, 11,000 IU vitamin A, 1,100 IU vitamin D₃, 22 IU vitamin E, 4 mg menadione as dimethylpyrimidinol bisulfate, 0.03 mg vitamin B₁₂, 28 mg d-pantothenic acid, 33 mg niacin, and 0.08% choline chloride.

²Calculated values.

³Analyzed values.

Abbreviations: ME, metabolizable energy; SID, standard ileal digestible.

diets with glutamate on the nitrogen balance or AA metabolism in the PDV and liver of pigs fed 3 diets (18% CP, 15% CP + 0.6% glutamate, and 13.5% CP + 1.8% glutamate). In present study, we just arranged 3 groups (18% CP, 15% CP + 0.6% calcium pyruvate, and 13.5% CP + 1.8% calcium pyruvate), not designing the groups of 15% CP + 0.6% glutamate and 13.5% CP + 1.8% glutamate, because it was difficult to perform these trials with 5 treatments at same time.

Eighteen barrows (40 ± 1.0 kg) were randomly assigned to receive 1 of 3 basal diets ($n = 6$ per treatment). The diets with 15.0% and 13.5% were supplemented with 0.6% and 1.8% calcium pyruvate, respectively. The experimental period lasted 14 d; habituation for the first 7 and sample collection for the remainder. From day 8 to day 14, TiO₂ (0.3%, as a digesta marker) was administered with feed. The feed daily supplied to each pig (45 g/kg body weight) was equally divided into 2 parts, given at 0700 and 1700. Urine and feces were collected at 0800, 1500, and 2300 from day 11 to day 14. The collected feces (approximately 0.2 kg per pig per collection time) were combined with 10 mL of 10% sulfuric acid per 100 g feces, and frozen at -20 °C. All urine samples were collected in plastic bottles, weighed, recorded, and stored at -20 °C. Five milliliters of toluene and 6 N HCl were added daily to each urine collection bottle, preventing evaporation and nitrogen loss. Feed intake was recorded daily during the sample collection period. At the end of the collection period, the urine and feces were pooled per pig, mixed, and subsampled. The feces subsamples were dried, ground, and passed through a 1-mm screen for nutrient analysis. All samples were analyzed in duplicate. The dry matter (982.30 E; AOAC, 2000) of feces and feed, CP (954.01; AOAC, 2000) in feed, diet, urine and feces and Ca (984.01; AOAC, 2006) and P (965.17; AOAC, 2006) content in feed were determined. The content of titanium in feed and feces was analyzed according to a previously published method with a slight modification that involved sample digestion for 4 h (Myers et al., 2004). Total nitrogen excretion (g/d) was calculated as the sum of urinary nitrogen excretion (g/d) and fecal nitrogen excretion (g/d); urinary nitrogen excretion (g/d) = volume of urine per day × nitrogen content in urine; fecal nitrogen excretion (g/d) = weight of feces per day × nitrogen content in feces. The difference between nitrogen intake (g/d) and total excretion of nitrogen (g/d) was deemed the nitrogen balance (g/d).

Nutrient Fluxes Across the Portal and Hepatic Veins

Following 24-h food deprivation, 15 barrows (40 ± 1.0 kg) were surgically fitted with permanent catheters in the mesenteric vein, portal vein, carotid artery, and hepatic vein (Yin et al., 2010; Li et al., 2015). To address the difficulties in installation of hepatic-vein catheter and blockage of catheters after surgery, we made the following modifications: 1) multi-port catheters were used instead of

single-port catheters; and 2) the common polyvinyl chloride catheter was replaced with a central venous catheter made of polyurethane. Pigs were administered an intramuscular injection of penicillin (1.6×10^7 units) twice daily for a week after surgery. After surgery, the catheters were flushed and filled with aseptic saline solution (200 IU heparin/mL) twice daily. All animals were allowed to recover for at least 7 d or until achieving >85% of their presurgery feed intake for ≥ 2 d.

After recovery from surgery, the barrows were randomly assigned to receive 1 of 3 basal diets (Table 1; $n = 5$ per treatment). The diets with 15.0% and 13.5% were supplemented with 0.6% and 1.8% calcium pyruvate, respectively. The experimental period last 7 d. The pigs were fed 15 g/kg body weight at 0800, 1400, and 2000 throughout the experimental period including the day of blood sampling. On the last day, a priming dose (15 mL) of *p*-aminohippuric acid (PAH; Serva GmbH, Heidelberg, Baden-Württemberg, Germany) solution (15 mg/mL) was administered through the mesenteric vein at 0730 h, followed by a constant infusion of PAH solution at a rate of 0.8 mL/min. Sixty minutes after the priming dose, 5 mL blood samples per pig were collected from the portal vein, carotid artery, and hepatic vein at 0830, 1000, 1200, 1430, and 1730 h. Sodium heparin solution (100 IU/mL) was used as an anticoagulant. Samples were cooled on ice and transferred to the laboratory for centrifugation at $1,500 \times g$ at 4°C for 20 min. The harvested plasma was stored at -20°C until needed to measure PAH, AA, urea, and NH_3 .

The concentration of plasma PAH was determined according to the method described previously (Yin et al., 2010; Li et al., 2015). The portal vein plasma flow (PVPF; mL/min) and hepatic vein plasma flow (HVPF; mL/min) were calculated with the following equations:

$$\text{PVPF} = C_i \times \text{IR} / (\text{PAH}_{\text{pv}} - \text{PAH}_a);$$

$$\text{HVPF} = C_i \times \text{IR} / (\text{PAH}_{\text{hv}} - \text{PAH}_a);$$

where C_i is the concentration of infused PAH solution (mg/mL); IR is the infusion rate (mL/min) of PAH; and PAH_{pv} , PAH_{hv} , and PAH_a are the PAH concentrations (mg/mL) in the portal vein, hepatic vein, and carotid artery, respectively.

Hepatic artery plasma flow (HAPF; mL/min) = HVPF (mL/min) – HVPF (mL/min).

For free AA analysis, the frozen plasma samples were thawed at 4°C . The protein precipitation in

plasma was carried out as followed procedures. Briefly, 1 mL of the plasma sample was mixed with 2.5 mL of 7.5% (w/v) trichloroacetic acid solution and the mixture was centrifuged at $12,000 \times g$ and 4°C for 15 min. The supernatant was collected and analyzed for AA by gas chromatography-mass spectrometer (GC-MS) according to the isotope dilution method described by Calder et al. (1999). The analysis for NH_3 needed to be performed within 2 h after blood collection. The procedures for determining urea and NH_3 have been described previously (Zhu et al., 2000). The fluxes of nitrogen-containing compounds across the portal vein, carotid artery, and hepatic vein were calculated according to the methods described previously (Yin et al., 2010; Li et al., 2015). The differences in the fluxes of nitrogen-containing compounds across the portal vein and the net portal fluxes of nitrogen-containing compounds were emphasized. The former is the product of the plasma flow rate across the portal vein and the concentration of plasma nitrogen-containing compound in the portal vein. The latter is the product of PVPF and difference in plasma nitrogen-containing compound between portal vein and carotid artery. The consumption rate of AAs in the liver (mg/min) = AA concentration in hepatic artery (mg/mL) $\times$ HAPF (mL/min) + AA concentration in portal vein (mg/mL) $\times$ PVPF (mL/min) – AA concentration in hepatic vein (mg/mL) $\times$ HVPF (mL/min). What need to indicate is that the concentrations of AA in hepatic artery and carotid artery are the same (composition of arterial blood is essentially the same no matter the sampling site), and that's why the catheter was inserted at the carotid artery. The production rate of urea in the liver (mg/min) = urea concentration in hepatic vein (mg/mL) $\times$ HVPF (mL/min) – urea concentration in hepatic artery (mg/mL) $\times$ HAPF

A

Figure 1. The rate of ^{14}C glutamate and ^{14}C pyruvate oxidation to $^{14}\text{CO}_2$ in cells when sodium pyruvate content in medium increased from 0 mM to 400 mg/liter (%). $n = 6$ each. All data are mean $\pm$ SD; a,b Values with different letter superscripts within the same index mean significant difference ($P < 0.05$).

Table 2. The effects of addition of calcium pyruvate to low-protein diets on the nitrogen balance in pigs

	Treatments ¹			SEM	<i>P</i>
	I	II	III		
Intake of N (g/d)	44.5 ^a	39.1 ^b	35.4 ^c	0.66	<0.001
Fecal N (g/d)	9.31 ^a	7.49 ^b	6.36 ^c	0.18	<0.001
Urinary N (g/d)	13.2 ^a	10.3 ^b	7.80 ^c	0.20	<0.001
Total N excretion (g/d)	22.5 ^a	17.8 ^b	14.2 ^c	0.37	<0.001
Retention of N (g/d)	22.0	21.3	21.2	0.36	0.399
Fecal N/N intake (%)	20.9 ^a	19.2 ^b	18.0 ^c	0.19	<0.001
Urinary N/N intake (%)	29.7 ^a	26.3 ^b	22.0 ^c	0.21	<0.001
Total N excretion/N intake (%)	50.6 ^a	45.5 ^b	40.0 ^c	0.34	<0.001
Fecal N/N excretion (%)	41.4 ^a	42.1 ^{ab}	44.9 ^b	0.25	<0.001
Urinary N/N excretion (%)	58.6 ^a	57.9 ^{ab}	55.1 ^b	0.25	<0.001
Retention of N/N intake (%)	49.4 ^a	54.5 ^b	60.0 ^c	0.34	<0.001

Data are presented as mean $\pm$ SEM ($n = 6$). SEM = standard error of means.

¹I, control group (18% CP); II, group of 15.0% CP + 0.6% calcium pyruvate; III, group of 13.5% CP + 1.8% pyruvate.

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

Table 3. The plasma flows across portal vein, hepatic artery, and hepatic vein in pigs

	Treatments ¹			SEM	<i>P</i>
	I	II	III		
PVPF (mL/min)	1,085	1,077	1,057	11.3	0.496
HAPF (mL/min)	281	294	317	8.3	0.805
HVPF (mL/min)	1,366	1,370	1,374	8.8	0.142

Data are presented as mean $\pm$ SEM ($n = 6$). SEM = standard error of means; PVPF, HAPF, and HVPF are the plasma flow across portal vein, hepatic artery, and hepatic vein in pigs, respectively.

¹I, control group (18% CP); II, group of 15.0% CP + 0.6% calcium pyruvate; III, group of 13.5% CP + 1.8% pyruvate.

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

(mL/min) – urea concentration in portal vein (mg/mL) $\times$ PVPF (mL/min).

Statistical Analysis

Data (including nitrogen intake, urinary and fecal nitrogen excretion, nitrogen retention, concentrations of plasma nitrogen compounds in portal vein, hepatic artery, and hepatic vein, fluxes of nitrogen compounds across the corresponding vessels, net portal flux of nitrogen compounds, AA consumption rate in the liver, and production rate of urea in the liver) were subjected to the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC, USA). The model used was:

$$Y_{ij} = \mu + T_i + A_j + e_{ij}$$

where Y_{ij} = dependent variable, μ = overall mean, T_i = treatment, A_j = animal, and e_{ij} = residual error. Differences between treatment means were determined by Tukey's multiple comparison test. Results

are reported as means $\pm$ SEM and were considered statistically significant at $P < 0.05$.

To detect differences in the changes of glutamate and pyruvate oxidation by cells when sodium pyruvate content in medium increased from 110 mM to 400 mM, independent-samples Student's *t* test was used. Results are reported as least squares means and SEM values, and statistical significance was declared at $P < 0.05$.

RESULTS

The rate of [¹⁴C]Glu oxidation rate to CO₂ by IPEC-J2 cells was markedly decreased when the content of sodium pyruvate in culture medium was increased from 110 to 400 mM ($P < 0.05$) (Fig. 1).

The results of nitrogen excretion were presented in Table 2. Compared with the 18.0% CP diet (normal), addition of calcium pyruvate to low-protein diets that balanced lysine, threonine, methionine, and tryptophan significantly reduced urinary nitrogen excretion from 13.2 g/d (18% CP) to 10.3 g/d (15.0% CP) or 7.80 g/d (13.5% CP) and total nitrogen excretion from 22.5 g/d to 17.8 g/d (15.0% CP) or 14.2 g/d (13.5% CP) ($P < 0.05$), without obviously negative effects on the nitrogen retention ($P > 0.05$). The reduction of dietary CP content reduced the fecal nitrogen/nitrogen intake ratio, urinary nitrogen/nitrogen intake ratio, total nitrogen excretion/nitrogen intake ratio ($P < 0.05$) meanwhile increased the nitrogen retention/nitrogen intake ratio ($P < 0.05$).

The plasma flows across portal vein, hepatic artery, and hepatic vein in pigs were presented in Table 3. There were no differences in PVPF, HAPF, and HVPF among 3 groups ($P > 0.05$).

The concentrations of plasma nitrogen-containing compounds in the portal vein, hepatic artery, and hepatic vein are presented in Table 4. When compared with control diet (18% CP), addition of calcium pyruvate to the diet with 15% and 13.5% CP content decreased the concentration of plasma NH_3 in the portal vein ($P < 0.05$). In addition, the concentrations of plasma glycine and alanine in the portal vein of pigs fed the diet with 13.5% CP + 1.8% pyruvate were lower than those of pigs fed control diet ($P < 0.05$). There were no differences in the concentrations of plasma nitrogen compounds in the hepatic artery among 3 groups ($P > 0.05$). The concentration of plasma urea in the hepatic vein of pigs fed control diet was higher than that of pigs fed the diet with 13.5% CP + 1.8% pyruvate ($P < 0.05$).

The fluxes of plasma nitrogen-containing compounds across the portal vein, hepatic artery, and hepatic vein are presented in Table 5. When compared with control diet, addition of calcium pyruvate to low-protein diets reduced the fluxes of plasma glycine, alanine, and NH_3 across the portal

vein of pigs ($P < 0.05$). The flux of plasma urea across the hepatic vein of pigs fed the diet with 13.5% CP + 1.8% pyruvate was lower than that of pigs fed diet with 18% CP ($P > 0.05$). There were no differences in the fluxes of other determined plasma nitrogen compounds across hepatic artery and hepatic artery vein among 3 groups ($P > 0.05$).

The net portal fluxes of nitrogen-containing compounds were presented in Table 6. Addition of calcium pyruvate to low-protein diets reduced the net portal fluxes of plasma NH_3 , glycine, alanine, and NEAA meanwhile increased the net portal flux of plasma glutamate ($P < 0.05$). The consumption of nitrogen-containing compounds after crossing the liver of pigs was presented in Table 7. When compared to control diet, the diet with 15.0% CP + 0.6% calcium pyruvate decreased the consumption of glycine, alanine, NH_3 , NEAA, and total AA (TAA) after crossing the liver of pigs ($P < 0.05$) and the production rate of urea in the liver ($P < 0.05$); the diet with 13.5% CP + 1.8% calcium pyruvate reduced the consumption of threonine, valine, methionine, isoleucine, phenylalanine, proline, glycine, alanine, NH_3 , EAA, NEAA,

Table 4. The effects of addition of pyruvate to low-protein diets on the concentrations of nitrogen-containing compounds in the portal vein, hepatic artery, and hepatic vein of 40-kg pigs (mg/dL)¹

	Portal vein					Hepatic artery					Hepatic vein				
	I	II	III	SEM	P	I	II	III	SEM	P	I	II	III	SEM	P
Thr	3.91	3.81	3.76	0.17	0.822	3.25	3.25	3.22	0.13	0.978	3.65	3.58	3.56	0.13	0.876
Val	3.71	3.63	3.59	0.16	0.866	2.87	2.83	2.83	0.11	0.965	3.43	3.37	3.35	0.12	0.885
Met	1.35	1.32	1.26	0.06	0.543	0.89	0.90	0.83	0.04	0.727	1.12	1.11	1.05	0.04	0.870
Ile	2.21	2.15	2.05	0.09	0.508	1.58	1.46	1.39	0.06	0.206	1.93	1.86	1.78	0.07	0.419
Leu	3.43	3.34	3.34	0.15	0.883	2.90	2.79	2.72	0.11	0.672	3.11	3.03	3.00	0.11	0.793
Phe	2.70	2.62	2.61	0.12	0.850	2.00	1.98	1.89	0.08	0.823	2.40	2.36	2.32	0.08	0.847
Lys	3.32	3.24	3.22	0.14	0.878	2.43	2.36	2.33	0.10	0.865	2.94	2.88	2.86	0.10	0.862
His	2.23	2.17	2.15	0.10	0.834	1.62	1.60	1.51	0.06	0.701	1.99	1.95	1.90	0.07	0.779
Arg	3.15	3.07	3.07	0.14	0.903	2.38	2.30	2.22	0.09	0.668	2.78	2.70	2.67	0.10	0.770
Trp	0.57	0.58	0.54	0.01	0.516	0.41	0.42	0.40	0.01	0.524	0.46	0.46	0.42	0.02	0.537
Pro	2.67	2.57	2.54	0.11	0.706	2.06	1.90	1.80	0.08	0.152	2.38	2.28	2.24	0.08	0.600
Asp	1.17	1.17	1.14	0.05	0.960	0.95	0.96	0.93	0.04	0.941	1.21	1.24	1.21	0.04	0.804
Ser	2.02	1.98	1.89	0.09	0.641	1.73	1.70	1.60	0.07	0.280	1.89	1.87	1.75	0.07	0.364
Glu	4.38	4.42	4.41	0.20	0.588	4.79	4.78	4.77	0.19	0.995	5.18	5.15	5.18	0.18	0.999
Gly	5.40 ^a	4.91 ^{ab}	4.60 ^b	0.22	0.048	3.37	3.31	3.28	0.13	0.941	4.32	4.24	4.12	0.15	0.661
Ala	5.68 ^a	5.15 ^{ab}	4.72 ^b	0.23	0.034	3.52	3.56	3.33	0.14	0.588	4.43	4.33	4.16	0.15	0.487
Cys	1.89	1.85	1.81	0.08	0.781	1.62	1.55	1.53	0.06	0.397	1.78	1.73	1.68	0.06	0.445
Tys	2.23	2.27	2.22	0.10	0.993	2.02	2.02	1.99	0.08	0.942	2.10	2.12	2.07	0.07	0.851
EAA	26.7	26.2	25.6	1.23	0.821	20.3	19.9	19.2	0.85	0.783	23.8	23.3	23.1	0.90	0.808
NEAA	25.5	24.5	23.4	0.96	0.486	20.1	19.8	19.2	0.71	0.874	23.3	22.9	22.6	0.73	0.760
TAA	52.2	50.7	49.0	2.19	0.682	40.4	39.6	38.5	1.56	0.824	47.0	46.2	45.7	1.64	0.799
NH_3	0.91 ^a	0.73 ^b	0.65 ^b	0.04	0.007	0.47	0.44	0.42	0.02	0.226	0.43	0.39	0.37	0.02	0.454
Urea	7.61	7.47	7.34	0.33	0.876	7.85	7.72	7.58	0.31	0.887	8.52 ^a	8.28 ^{ab}	8.15 ^b	0.23	0.047

Data are presented as mean $\pm$ SEM ($n = 5$). EAA = essential amino acids; NEAA = non-essential amino acids; SEM = standard error of means; TAA = total amino acids.

¹I, control group (18% CP); II, group of 15.0% CP + 0.6% calcium pyruvate; III, group of 13.5% CP + 1.8% calcium pyruvate.

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

Table 5. The effects of addition of pyruvate to low-protein diets on the fluxes of nitrogen-containing compounds across the portal vein, hepatic artery, and hepatic vein of 40-kg pigs (mg/min)¹

	Portal vein					Hepatic artery					Hepatic vein				
	I	II	III	SEM	P	I	II	III	SEM	P	I	II	III	SEM	P
Thr	42.5	41.4	39.6	1.45	0.331	9.16	9.47	10.0	0.33	0.936	49.6	49.0	49.1	2.18	0.688
Val	40.4	39.5	37.8	1.39	0.382	8.04	8.31	8.88	0.29	0.985	46.6	46.0	45.6	2.04	0.694
Met	14.8	14.6	13.3	0.50	0.134	2.48	2.62	2.62	0.09	0.548	15.3	15.3	14.4	0.68	0.641
Ile	24.1	23.5	21.6	0.82	0.117	4.43	4.24	4.41	0.16	0.173	26.3	25.6	24.4	1.14	0.329
Leu	37.4	36.3	35.2	1.28	0.424	8.13	8.20	8.65	0.29	0.677	42.5	41.5	40.7	1.85	0.624
Phe	29.4	28.6	27.4	1.00	0.356	5.60	5.80	6.01	0.20	0.787	32.7	32.4	31.6	1.43	0.606
Lys	36.1	35.2	33.9	1.23	0.400	6.80	6.87	7.40	0.24	0.908	40.2	39.6	38.7	1.76	0.670
His	24.3	23.7	22.6	0.83	0.355	4.54	4.65	4.79	0.16	0.656	27.1	26.8	26.0	1.19	0.597
Arg	34.3	33.4	32.5	1.18	0.450	6.66	6.75	7.05	0.24	0.663	37.9	37.1	36.3	1.66	0.601
Trp	6.19	6.25	5.81	0.20	0.323	1.15	1.23	1.27	0.06	0.384	6.28	6.31	5.96	0.20	0.377
Pro	29.1	28.0	26.7	0.99	0.230	5.78	5.53	5.72	0.20	0.129	32.5	31.5	30.7	1.40	0.473
Asp	12.5	12.8	12.1	0.45	0.600	2.67	2.79	2.96	0.10	0.902	16.5	16.9	16.6	0.75	0.606
Ser	21.9	21.7	20.0	0.75	0.175	4.84	4.95	5.08	0.18	0.236	25.8	25.7	24.0	1.13	0.284
Glu	47.6	47.6	46.7	1.74	0.827	13.4	14.2	13.8	0.49	0.990	70.8	70.8	70.4	3.13	0.873
Gly	58.7 ^a	52.2 ^b	48.5 ^b	1.88	0.006	9.45	9.72	9.61	0.34	0.972	58.9	58.2	55.7	2.58	0.493
Ala	61.8 ^a	55.8 ^b	49.7 ^b	1.95	0.003	9.86	10.4	9.73	0.36	0.469	60.5	59.3	56.3	2.64	0.368
Cys	20.7	19.9	19.2	0.70	0.298	4.53	4.55	4.43	0.16	0.390	24.3	23.4	22.7	1.05	0.362
Tys	24.4	24.5	23.4	0.85	0.680	5.65	5.92	6.32	0.21	0.918	28.7	29.1	27.9	1.28	0.648
EAA	289	282	271	10.6	0.324	57.0	58.3	60.9	2.21	0.770	324	319	313	1.5	0.617
NEAA	277	264	247	8.2	0.106	56.2	58.1	61.1	1.84	0.855	318	314	305	12.5	0.550
TAA	566	546	518	18.9	0.204	113	116	122	4.1	0.833	642	633	619	27.8	0.592
NH ₃	9.84 ^a	7.88 ^b	6.84 ^b	0.30	<0.001	1.30	1.28	1.34	0.05	0.226	5.95	5.35	4.96	0.27	0.649
Urea	82.6	80.5	77.5	2.84	0.383	22.0	22.5	22.1	0.80	0.896	117 ^a	113 ^{ab}	109 ^b	4.85	0.035

Data are presented as mean $\pm$ SEM ($n = 5$). EAA = essential amino acids; NEAA = non-essential amino acids; SEM = standard error of means; TAA = total amino acids.

¹I, control group (18% CP); II, group of 15.0% CP + 0.6% calcium pyruvate; III, group of 13.5% CP + 1.8% calcium pyruvate.

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

and TAA after crossing the liver ($P < 0.05$) and the production rate of urea in the liver ($P < 0.05$).

DISCUSSION

In this study, we found that culturing cells with pyruvate reduced glutamate oxidation. The results suggest that pyruvate can substitute the role of glutamate acting as metabolic fuel at the cellular level. Therefore, pyruvate has the potential to reduce nitrogen through improving AA use efficiency.

In general, the dietary CP content can drop by 2% to 3% without affecting average daily weight gain in pigs (Tuitoek et al., 1997; Lu et al., 2008; Hernández et al., 2011). However, a further reduction in the dietary CP content would lower nitrogen retention and growth performance, even if the diet is supplemented with EAA (Figueroa et al., 2002; He et al., 2016). In this result, there were no differences in nitrogen retention among 3 groups (18% CP, 15% CP + 0.6% calcium pyruvate, and 13.5% CP + 1.8% calcium pyruvate). The results indicated that dietary pyruvate supplementation

could further decline the threshold of dietary CP content which does not affect porcine nitrogen retention. In comparison with pigs fed control diet with 18% CP, urinary nitrogen excretion, fecal nitrogen excretion, and total nitrogen excretion in pigs fed diet with 15% CP + 0.6% calcium pyruvate or with 13.5% CP + 1.8% calcium pyruvate were reduced 22.0%, 19.6%, and 21.0% or 40.9%, 31.6%, and 37.1%, respectively. In one our previous study, when compared to the pigs fed control diet with 18% CP, urinary nitrogen excretion, fecal nitrogen excretion, and total nitrogen excretion in pigs fed diet with 15% CP + 0.6% glutamate or with 13.5% CP + 1.8% glutamate were reduced 15.5%, 24.7%, and 19.1% or 19.3%, 31.6%, and 24.2%, respectively (Xu, 2017). It is obvious that the decreased proportion of total nitrogen excretion especially urinary excretion by pyruvate supplementation was much higher than that caused by glutamate supplement. These results indicate that pyruvate addition markedly reduced porcine nitrogen excretion in which urinary nitrogen accounts for most of the proportion through improving AA utilization.

Table 6. The net portal fluxes of nitrogen-containing compounds of pigs fed diets with different CP content (mg/min)

	Treatments ¹			SEM	P
	I	II	III		
Thr	7.04	6.54	6.37	0.36	0.402
Val	9.29	8.99	8.44	0.41	0.326
Met	5.11	4.86	4.59	0.20	0.291
Ile	6.94	7.82	7.04	0.31	0.156
Leu	5.90	6.26	6.55	0.33	0.397
Phe	7.67	7.27	7.56	0.33	0.605
Lys	9.78	9.78	9.44	0.42	0.747
His	6.72	6.54	6.83	0.29	0.707
Arg	8.47	8.66	9.01	0.38	0.679
Trp	1.74	1.72	1.58	0.07	0.944
Pro	6.72	7.55	7.82	0.32	0.091
Asp	2.10	2.46	2.34	0.12	0.082
Ser	3.25	3.37	3.32	0.18	0.930
Glu	-4.45 ^a	-3.82 ^b	-3.39 ^c	0.18	0.006
Gly	22.2 ^a	17.6 ^b	14.0 ^c	0.74	<0.001
Ala	23.6 ^a	17.5 ^b	14.7 ^c	0.77	<0.001
Cys	3.07	3.20	3.04	0.17	0.911
Tys	2.43	2.78	2.50	0.18	0.448
EAA	68.7	68.4	67.4	3.09	0.955
NEAA	58.9 ^a	50.6 ^b	43.8 ^b	2.51	0.004
TAA	128	119	111	5.1	0.091
NH ₃	4.80 ^a	3.15 ^b	2.37 ^c	0.12	<0.001
Urea	-2.61	-2.69	-2.51	1.47	0.994

Data are presented as mean $\pm$ SEM ($n = 5$). EAA = essential amino acids; NEAA = non-essential amino acids; SEM = standard error of means; TAA = total amino acids.

¹I, control group (18% CP); II, group of 15.0% CP + 0.6% calcium pyruvate; III, group of 13.5% CP + 1.8% calcium pyruvate.

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

The changes of AA metabolism in the PDV and liver in response to addition of pyruvate to low-protein diets revealed the inner mechanisms for the enhanced AA use efficiency and decreased nitrogen excretion. Specifically, the net portal fluxes of NEAA in pigs fed calcium pyruvate-supplemented low-protein diets and the net portal fluxes of TAA in pigs fed diet with 13.5% CP + 1.8% calcium pyruvate were lower than those of pigs fed control diet with 18% CP. However, the consumption rate of EAA in the liver of pigs fed diet with 13.5% CP + 1.8% calcium pyruvate and the consumption rate of NEAA in the liver of pigs fed calcium pyruvate-supplemented low-protein diets were lower than those of pigs fed control diet. The above results provided a possible explanation for the fact that there were no differences in the total numbers of EAA, NEAA, and TAA supplied to extra-hepatic tissues among control diet and pyruvate-supplemented low-protein diets.

Table 7. The changes of nitrogen-containing compounds after crossing the liver of pigs fed diets with different CP content (mg/min)

	Treatments			SEM	P
	I	II	III		
Thr	2.08 ^a	1.90 ^a	0.70 ^b	0.16	0.001
Val	1.86 ^a	1.79 ^a	1.28 ^b	0.14	0.014
Met	2.03 ^a	1.98 ^a	1.50 ^b	0.07	<0.001
Ile	2.22 ^a	2.27 ^a	1.72 ^b	0.10	0.003
Leu	3.05	3.04	3.12	0.13	0.194
Phe	2.26 ^a	2.06 ^{ab}	1.82 ^b	0.11	0.023
Lys	2.74	2.61	2.47	0.14	0.057
His	1.71	1.76	1.49	0.09	0.090
Arg	3.01	3.10	3.13	0.13	0.405
Trp	1.05	1.18	1.12	0.03	0.098
Pro	2.41 ^a	2.34 ^{ab}	2.00 ^b	0.11	0.035
Asp	-1.36	-1.38	-1.49	0.08	0.460
Ser	1.02	1.05	1.19	0.08	0.159
Glu	-9.77	-8.97	-8.56	0.43	0.402
Gly	9.29 ^a	4.79 ^b	3.09 ^c	0.29	<0.001
Ala	11.1 ^a	7.00 ^b	3.82 ^c	0.32	<0.001
Cys	1.08	1.17	1.12	0.07	0.096
Tys	1.33	1.38	1.49	0.08	0.482
EAA	22.0 ^a	21.7 ^a	18.4 ^b	1.06	0.029
NEAA	14.9 ^a	7.37 ^b	2.71 ^c	1.05	<0.001
TAA	37.0 ^a	29.1 ^b	21.1 ^c	2.05	<0.001
NH ₃	5.23 ^a	3.83 ^b	3.22 ^c	0.20	<0.001
Urea	-12.6 ^a	-10.1 ^b	-7.83 ^c	0.35	<0.001

Data are presented as mean $\pm$ SEM ($n = 5$). EAA = essential amino acids; NEAA = non-essential amino acids; SEM = standard error of means; TAA = total amino acids.

¹I, control group (18% CP); II, group of 15.0% CP + 0.6% calcium pyruvate; III, group of 13.5% CP + 1.8% calcium pyruvate.

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

Of importance is that dietary pyruvate supplementation reduced the net portal fluxes of glycine, alanine, and NH₃. Our previous work has demonstrated that glycine and alanine as well as NH₃ are the main nitrogen sources of urea (Yang, 2016), which is regulatory target to address livestock nitrogen pollution, given that urinary nitrogen accounts for >50% of total nitrogen excretion (Patr et al., 2012; Shiral et al., 2012). The results in present study indicated that supplementing low-protein diets with calcium pyruvate reduced the release of nitrogen precursors of urea to the liver. As a result, the production rate of urea in pigs fed calcium pyruvate-supplemented diets was reduced. The decrease of urea production should be one primary reason for the decreased urinary nitrogen in this study.

Pyruvate is popular for being as a human dietary supplement. The biological functions of pyruvate include: 1) inducing weight loss via increased

metabolism in muscle tissue (Koh-Banerjee et al., 2005); 2) enhancing physical endurance during rest or exercise, by increasing blood glucose extraction from exercising muscle (Stanko et al., 1990); 3) effectively reducing cholesterol (Stanko et al., 1994); (4) being as is an effective antioxidant (Borle and Stanko, 1996). At present, the information about effects of addition of pyruvate to low-protein diets on the AA metabolism and nitrogen excretion in pigs is limited. As with glutamate, these benefits of pyruvate should be due to its pivotal role in numerous metabolic pathways (Bricker et al., 2012), acting as a master fuel input undergirding citric acid cycle carbon flux (Gray et al., 2014), and consequently substituting the role of AA (e.g., glutamate) as metabolic fuel and reducing nitrogen excretion in pigs.

Unfortunately, pyruvate's high price precludes widespread use in pig production. Thus, to increase the applicability of our findings, it may be necessary to target methods of increasing pyruvate entry and oxidation in mitochondria. In mitochondria, pyruvate drives ATP production by oxidative phosphorylation and multiple biosynthetic pathways intersecting the citric acid cycle. The process is regulated by many enzymes, including the recently discovered mitochondria pyruvate carrier, pyruvate dehydrogenase (PDH), and pyruvate carboxylase, to modulate overall pyruvate carbon flux. Among these, PDH contributes strongly to flux control of myocardial glucose oxidation (Randle, 1986; Patel and Roche, 1990; Randle et al., 1994; Priestman et al., 1996). We recommend that future studies focus on activating PDH and promoting the glucose-pyruvate oxidation pathway. Doing so should inhibit the use of AA as metabolic fuel and consequently reduce nitrogen excretion in pigs.

In conclusion, this study observed that culturing cells with pyruvate reduced glutamate oxidation. Supplementing low-protein diets with pyruvate reduced the release of nitrogen precursors of urea in the PDV and the consumption rate of AA in the liver and increased the supply of AA to extra-hepatic tissues. Consequently, supplementing low-protein diets (15% CP and 13.5% CP) with pyruvate reduced production rate of urea and nitrogen excretion especially urinary nitrogen excretion without obvious negative impacts on nitrogen retention in pigs.

ACKNOWLEDGMENTS

We gratefully acknowledge the financial support from the National Natural Science Foundation of China (31772610), the National "948" Project from the Ministry of Agriculture of China (2015Z74), and the National Science and Technology Major

Project of China (2017YFD0500504). We thank Zhengya Liu, Yong Fang, Xiaoqiang Xue, and Dandan Zhang for technical support during the catheter surgery. All authors read and approved the final manuscript.

LITERATURE CITED

- Abe, T., S. Takahashi, and N. Suzuki. 2006. Oxidative metabolism in cultured rat astroglia: effects of reducing the glucose concentration in the culture medium and of D-aspartate or potassium stimulation. *J. Cereb. Blood Flow Metab.* 26:153–160. doi:10.1038/sj.jcbfm.9600175
- AOAC. 2000. Official methods of analysis. 17th ed. Association of Official Analytical Chemists, Washington, DC.
- AOAC. 2006. Official methods of analysis. 18th ed. Association of Official Analytical Chemists, Arlington, VA.
- Borle, A. B., and R. T. Stanko. 1996. Pyruvate reduces anoxic injury and free radical formation in perfused rat hepatocytes. *Am. J. Physiol.* 270:G535–G540. doi:10.1152/ajpgi.1996.270.3.G535
- Bricker, D. K., E. B. Taylor, J. C. Schell, T. Orsak, A. Boutron, Y. C. Chen, J. E. Cox, C. M. Cardon, J. G. Van Vranken, N. Dephore, et al. 2012. A mitochondrial pyruvate carrier required for pyruvate uptake in yeast, *Drosophila*, and humans. *Science* 337:96–100. doi:10.1126/science.1218099
- Calder, A. G., K. E. Garden, S. E. Anderson, and G. E. Lobley. 1999. Quantitation of blood and plasma amino acids using isotope dilution electron impact gas chromatography/mass spectrometry with U-13C amino acids as internal standards. *Rapid Commun. Mass Spectrom.* 13:2080–2083.
- Chen, C. 2015. Study for effects of dietary protein levels on amino acid metabolism and transformation in the liver of piglets[D]. Master thesis, Southwest University, Chongqing, China.
- Figuerola, J. L., A. J. Lewis, P. S. Miller, R. L. Fischer, R. S. Gómez, and R. M. Diedrichsen. 2002. Nitrogen metabolism and growth performance of gilts fed standard corn-soybean meal diets or low-crude protein, amino acid-supplemented diets. *J. Anim. Sci.* 80:2911–2919. doi:10.2527/2002.80112911x
- Galassi, G., S. Colombini, L. Malagutti, G. M. Crovetto, and L. Rapetti. 2010. Effects of high fibre and low protein diets on performance, digestibility, nitrogen excretion and ammonia emission in the heavy pig. *Anim. Feed Sci. Tech.* 16:1140–1148. doi:10.1016/j.anifeedsci.2010.08.009
- Gallo, L., G. D. Montà, L. Carraro, A. Cecchinato, P. Carnier, and S. Schiavon. 2014. Growth performance of heavy pigs fed restrictively diets with decreasing crude protein and indispensable amino acids content. *Livest. Sci.* 161:130–138. doi:10.1016/j.livsci.2013.12.027
- Gray, L. R., M. R. Sultana, A. J. Rauckhorst, L. Oonthonpan, S. C. Tompkins, A. Sharma, X. Fu, R. Miao, A. D. Pawa, K. S. Brown, et al. 2015. Hepatic mitochondrial pyruvate carrier 1 is required for efficient regulation of gluconeogenesis and whole-body glucose homeostasis. *Cell Metab.* 22:669–681. doi:10.1016/j.cmet.2015.07.027
- Gray, L. R., S. C. Tompkins, and E. B. Taylor. 2014. Regulation of pyruvate metabolism and human disease. *Cell. Mol. Life Sci.* 71:2577–2604. doi:10.1007/s00018-013-1539-2
- He, L., L. Wu, Z. Xu, T. Li, K. Yao, Z. Cui, Y. Yin, and G. Wu. 2016. Low-protein diets affect ileal amino acid digestibility

- and gene expression of digestive enzymes in growing and finishing pigs. *Amino Acids* 48:21–30. doi:10.1007/s00726-015-2059-1
- Hernández, F., S. Martínez, C. López, M. D. Megías, M. López, and J. Madrid. 2011. Effect of dietary crude protein levels in a commercial range, on the nitrogen balance, ammonia emission and pollutant characteristics of slurry in fattening pigs. *Animal* 5:1290–1298. doi:10.1017/S1751731111000115
- Hinson, R. B., A. P. Schinckel, J. S. Radcliffe, G. L. Allee, A. L. Sutton, and B. T. Richert. 2009. Effect of feeding reduced crude protein and phosphorus diets on weaning-finishing pig growth performance, carcass characteristics, and bone characteristics. *J. Anim. Sci.* 87:1502–1517. doi:10.2527/jas.2008-1325
- Hong, J. S., G. I. Lee, X. H. Jin, and Y. Y. Kim. 2016. Effect of dietary energy levels and phase feeding by protein levels on growth performance, blood profiles and carcass characteristics in growing-finishing pigs. *J. Anim. Sci. Technol.* 58:37. doi:10.1186/s40781-016-0119-z
- Kerr, B. J., and R. A. Easter. 1995. Effect of feeding reduced protein, amino acid-supplemented diets on nitrogen and energy balance in grower pigs. *J. Anim. Sci.* 73:3000–3008.
- Kerr, B. J., L. L. Southern, T. D. Bidner, K. G. Friesen, and R. A. Easter. 2003. Influence of dietary protein level, amino acid supplementation, and dietary energy levels on growing-finishing pig performance and carcass composition. *J. Anim. Sci.* 81:3075–3087. doi:10.2527/2003.81123075x
- Koh-Banerjee, P. K., M. P. Ferreira, M. Greenwood, R. G. Bowden, P. N. Cowan, A. L. Almada, and R. B. Kreider. 2005. Effects of calcium pyruvate supplementation during training on body composition, exercise capacity, and metabolic responses to exercise. *Nutrition* 21:312–319. doi:10.1016/j.nut.2004.06.026
- Le Bellego, L., J. van Milgen, and J. Noblet. 2002. Effect of high temperature and low-protein diets on the performance of growing-finishing pigs. *J. Anim. Sci.* 80:691–701. doi:10.2527/2002.803691x
- Li, Y., F. Li, Y. Duan, Q. Guo, W. Wang, C. Wen, X. Huang, and Y. Yin. 2017. The protein and energy metabolic response of skeletal muscle to the low-protein diets in growing pigs. *J. Agric. Food Chem.* 65:8544–8551. doi:10.1021/acs.jafc.7b02461
- Li, L., P. Zhang, P. Zheng, Z. Bao, Y. Wang, and F. R. Huang. 2015. Hepatic cumulative net appearance of amino acids and related gene expression response to different protein diets in pigs. *Livest. Sci.* 182:11–21. doi:10.1016/j.livsci.2015.10.012
- Lu, N., X. Yi, S. Qiao, and Q. Liu. 2008. The application of crystal amino acids in low protein diets of growing pigs. *Am. Sci.* 2:17–25.
- Luo, Z., C. Li, Y. Cheng, S. Hang, and W. Zhu. 2015. Effects of low dietary protein on the metabolites and microbial communities in the caecal digesta of piglets. *Arch. Anim. Nutr.* 69:212–226. doi:10.1080/1745039X.2015.1034521
- Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. Technical note: a procedure for the preparation and quantitative analysis of samples for titanium dioxide. *J. Anim. Sci.* 82:179–183. doi:10.2527/2004.821179x
- Newsholme, P., J. Procopio, M. M. Lima, T. C. Pithon-Curi, and R. Curi. 2003. Glutamine and glutamate—their central role in cell metabolism and function. *Cell Biochem. Funct.* 21:1–9. doi:10.1002/cbf.1003
- NRC. 2012. *Nutrient Requirements of Swine*. 11th ed. National Academies Press, Washington DC.
- Otto, E. R., M. Yokoyama, P. K. Ku, N. K. Ames, and N. L. Trottier. 2003. Nitrogen balance and ileal amino acid digestibility in growing pigs fed diets reduced in protein concentration. *J. Anim. Sci.* 81:1743–1753. doi:10.2527/2003.8171743x
- Patel, M. S., and T. E. Roche. 1990. Molecular biology and biochemistry of pyruvate dehydrogenase complexes. *FASEB J.* 4:3224–3233. doi:10.1096/fasebj.4.14.2227213
- Patrás, P., S. Nitrayová, M. Brestenský, and J. Heger. 2012. Effect of dietary fiber and crude protein content in feed on nitrogen retention in pigs. *J. Anim. Sci.* 90:158–160. doi:10.2527/jas.53837
- Priestman, D. A., K. A. Orfali, and M. C. Sugden. 1996. Pyruvate inhibition of pyruvate dehydrogenase kinase. Effects of progressive starvation and hyperthyroidism in vivo, and of dibutyl cyclic AMP and fatty acids in cultured cardiac myocytes. *FEBS Lett.* 393:174–178. doi:10.1016/0014-5793(96)00877-0
- Randle, P. J. 1986. Fuel selection in animals. *Biochem. Soc. Trans.* 14:799–806. doi:10.1042/bst0140799
- Randle, P. J., D. A. Priestman, S. Mistry, and A. Halsall. 1994. Mechanisms modifying glucose oxidation in diabetes mellitus. *Diabetologia* 37:S155–S161. doi:10.1007/BF00400839
- Reeds, P. J., D. G. Burrin, B. Stoll, and F. Jahoor. 2000. Intestinal glutamate metabolism. *J. Nutr.* 130:978S–982S. doi:10.1093/jn/130.4.978S
- Shirali, M., A. Doeschl-Wilson, P. W. Knap, C. Duthie, E. Kanis, J. A. van Arendonk, and R. Roehe. 2012. Nitrogen excretion at different stages of growth and its association with production traits in growing pigs. *J. Anim. Sci.* 90:1756–1765. doi:10.2527/jas.2011-4547
- Shriver, J. A., S. D. Carter, A. L. Sutton, B. T. Richert, B. W. Senne, and L. A. Pettet. 2003. 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.* 81:492–502. doi:10.2527/2003.812492x
- Stanko, R. T., H. R. Reynolds, R. Hoyson, J. E. Janosky, and R. Wolf. 1994. Pyruvate supplementation of a low-cholesterol, low-fat diet: effects on plasma lipid concentrations and body composition in hyperlipidemic patients. *Am. J. Clin. Nutr.* 59:423–427. doi:10.1093/ajcn/59.2.423
- Stanko, R. T., R. J. Robertson, R. W. Galbreath, J. J. Reilly, Jr, K. D. Greenawalt, and F. L. Goss. 1990. Enhanced leg exercise endurance with a high-carbohydrate diet and dihydroxyacetone and pyruvate. *J. Appl. Physiol.* 69:1651–1656. doi:10.1152/jappl.1990.69.5.1651
- Stoll, B., J. Henry, P. J. Reeds, H. Yu, F. Jahoor, and D. G. Burrin. 1998. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. *J. Nutr.* 128:606–614. doi:10.1093/jn/128.3.606
- Tuotoek, K., L. G. Young, C. F. de Lange, and B. J. Kerr. 1997. The effect of reducing excess dietary amino acids on growing-finishing pig performance: an elevation of the ideal protein concept. *J. Anim. Sci.* 75:1575–1583. doi:10.2527/1997.7561575x
- Vacanti, N. M., A. S. Divakaruni, C. R. Green, S. J. Parker, R. R. Henry, T. P. Ciaraldi, A. N. Murphy, and C. M. Metallo. 2014. Regulation of substrate utilization by

- the mitochondrial pyruvate carrier. *Mol. Cell* 56:425–435. doi:10.1016/j.molcel.2014.09.024
- Wu, Z. L. 2016. Study for the metabolic rules of amino acids in portal-drained viscera of pigs at different growth stages[D]. Master thesis, Southwest University, Chongqing, China.
- Xu, Q. Q. 2017. Study on the effects of balancing low-protein diets with glutamate on the utilization of protein and growth performance of finishing pigs[D]. Master thesis, Southwest University, Chongqing, China.
- Yang, J. 2016. Study for the metabolic fate of glycine and alanine in the porcine liver[D]. Master thesis, Southwest University, Chongqing, China.
- Yin, Y., R. Huang, T. Li, Z. Ruan, M. Xie, Z. Deng, Y. Hou, and G. Wu. 2010. Amino acid metabolism in the portal-drained viscera of young pigs: effects of dietary supplementation with chitosan and pea hull. *Amino Acids* 39:1581–1587. doi:10.1007/s00726-010-0577-4
- Zhang, S., L. Chu, S. Qiao, X. Mao, and X. Zeng. 2016. Effects of dietary leucine supplementation in low crude protein diets on performance, nitrogen balance, whole-body protein turnover, carcass characteristics and meat quality of finishing pigs. *Anim. Sci. J.* 87:911–920. doi:10.1111/asj.12520
- Zhu, L. H., L. E. Armentano, D. R. Bremmer, R. R. Grummer, and S. J. Bertics. 2000. Plasma concentration of urea, ammonia, glutamine around calving, and the relation of hepatic triglyceride, to plasma ammonia removal and blood acid-base balance. *J. Dairy Sci.* 83:734–740. doi:10.3168/jds.S0022-0302(00)74935-6