

## Amino acid metabolism in the portal-drained viscera of young pigs: effects of dietary supplementation with chitosan and pea hull

Yulong Yin · Ruilin Huang · Tiejun Li ·  
Zheng Ruan · Mingyong Xie · Zeyuan Deng ·  
Yongqing Hou · Guoyao Wu

Received: 6 August 2008 / Accepted: 18 March 2010 / Published online: 2 April 2010  
© Springer-Verlag 2010

**Abstract** Recent studies indicate extensive catabolism of amino acids (AA) by the portal-drained viscera (PDV) of pigs and humans. Because of ethical concerns over invasive surgical procedures on infants or adults, in vivo investigations are often performed with the pig which is both an agriculturally important livestock species and a widely used animal model for nutritional and physiological studies in humans. Here, we described a new technique for implanting chronic catheters into the portal vein, ileal mesenteric vein, and carotid artery to study AA metabolism in the PDV of young pigs. This method allowed for the reduction of surgery time by 1 h and measurements of the entry of dietary AA into the portal circulation. Using such an approach, we found that dietary supplementation with 100 mg/kg chitosan (a prebiotic and a polysaccharide not digested by animal cells) reduced oxygen consumption, as well as the net absorption of dietary AA into the portal vein, thereby enhancing their bioavailability for extraintestinal tissues. In contrast, opposite results were obtained

with dietary supplementation of 12% pea-hull (containing 95% of fermentable nonstarch polysaccharide). Thus, this improved technique is useful to quantify in vivo absorption and metabolism of dietary AA in young pigs.

**Keywords** Amino acids · Catheter implantation · Prebiotics · Pigs · Portal vein-drained viscera

### Abbreviations

|      |                              |
|------|------------------------------|
| AA   | Amino acids                  |
| BCAA | Branched-chain amino acids   |
| PAH  | Para-aminohippuric acid      |
| PVBF | Portal-vein blood flow rate  |
| PVPF | Portal-vein plasma flow rate |
| PDV  | Portal-drained viscera       |

### Introduction

The portal-drained viscera (PDV) consist of metabolically active organs, including the small intestine, pancreas, spleen, stomach, and part of the large intestine (Blachier et al. 2007, 2009; Burrin et al. 2000; Wang et al. 2009a). The small intestine represents approximately 70% of the PDV weight in young pigs (Deng et al. 2009; Tan et al. 2009a). In contrast to the traditional view that all of dietary amino acids (AA) absorbed from the small-intestine lumen entered the portal circulation, extensive investigations over the past two decades have indicated extensive catabolism of AA by the PDV of pigs and humans (Wu et al. 2005, 2009). For example, studies involving oral and intravenous administration of stable tracer AA led to the estimations that 97% of glutamate and aspartate, 70% of glutamine,

Y. Yin (✉) · R. Huang · T. Li  
Hunan Engineering and Research Center of Animal and Poultry Science, Key Laboratory for Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Hunan 410125, China  
e-mail: yinyulong@isa.ac.cn

Y. Yin · Z. Ruan · M. Xie · Z. Deng  
State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China

Y. Yin · Y. Hou  
Hubei Key Laboratory of Animal Nutrition and Feed Science, Wuhan Polytechnic University, Wuhan 430023, China

G. Wu  
Department of Animal Science, Texas A and M University, College Station, TX 77843, USA

40–50% of serine and glycine, 40% of arginine and proline, 20–40% of branched-chain AA (BCAA), 30–60% of other essential AA (lysine, methionine, phenylalanine, and threonine) in enteral diets were extracted in first pass by the small intestine (Stoll and Burrin 2006; Wu 1998). Additionally, in the post-absorptive pigs (Wu et al. 1994) and humans (Ligthart-Melis et al. 2008; van de Poll et al. 2007), the small intestine releases large amounts of citrulline, alanine, and proline, indicating their *de novo* synthesis by the gut. At present, direct quantification of AA metabolism in the PDV of conscious subjects is lacking because of technical difficulties in placing catheters into the portal vein and their maintenance for a prolonged period. Furthermore, ethical concerns over invasive surgical procedures on human subjects have precluded conduct of such studies in infants or adults.

Rerat et al. (1984) and Yen and Killefer (1987) developed methods to determine the entry of dietary sugars and nitrogenous substances into the portal vein of conscious pigs. Although these techniques have substantially advanced our knowledge of nutrient absorption, they have some major shortcomings, including (1) difficulties encountered while implanting portal and ileal mesenteric vein catheters; (2) a long period (3–4 h) required for the surgery; and (3) low survival rates for the animals. Here, we described a new technique for implanting chronic portal vein and ileal mesenteric vein catheters to study AA metabolism in the PDV of young pigs. The usefulness of this method was evaluated by assessing the effects of dietary supplementation with chitosan (a prebiotic and a polysaccharide not digested by animal cells) or pea hull (containing 95% fermentable nonstarch polysaccharide) on PDV oxygen consumption, as well as the entry of dietary AA and glucose into the porcine portal vein.

## Materials and methods

### Animals and diets

Fifteen male Durac × Landrace × Yorkshine pigs (initial body weight of  $15 \pm 1$  kg) were randomly allocated into one of three dietary groups ( $n = 5/\text{group}$ ): a cornstarch- and casein-based diet (Table 1); and the basal diet supplemented with 12% pea hull or 100 mg/kg of chitosan at the expense of cornstarch (Huang et al. 2007). This basal diet contained 1.85% L-glutamate plus L-glutamine (assumingly 1.11% L-glutamate plus 0.74% L-glutamine), 0.60% L-aspartate plus L-asparagine (assumingly 0.36% L-aspartate plus 0.24% L-asparagine), as well as supplemental AA (3.3% L-aspartic acid, 3.3% L-glutamic acid, 0.06% L-isoleucine, 0.23% L-methionine, and 0.07% L-tryptophan; Sigma Chemicals, St. Louis, MO, USA). The

**Table 1** Composition of experimental diets (as-fed basis)

| Ingredients         | Control diet | Chitosan diet | Pea-hull diet |
|---------------------|--------------|---------------|---------------|
| Corn starch         | 67.19        | 67.18         | 55.19         |
| Casein              | 8.5          | 8.5           | 8.5           |
| Chitosan            | 0.0          | 0.01          | 0.0           |
| Pea hull            | 0            | 0             | 12            |
| Sucrose             | 10           | 10            | 10            |
| Soybean oil         | 2            | 2             | 2             |
| Dicalcium phosphate | 2            | 2             | 2             |
| Potassium chloride  | 1.15         | 1.15          | 1.15          |
| Limestone           | 0.90         | 0.90          | 0.90          |
| Premix <sup>a</sup> | 0.6          | 0.6           | 0.6           |
| Magnesium sulfate   | 0.5          | 0.5           | 0.5           |
| Salt                | 0.2          | 0.2           | 0.2           |
| L-Aspartic acid     | 3.3          | 3.3           | 3.3           |
| L-Glutamic acid     | 3.3          | 3.3           | 3.3           |
| L-Isoleucine        | 0.06         | 0.06          | 0.06          |
| L-Methionine        | 0.23         | 0.23          | 0.23          |
| L-Tryptophan        | 0.07         | 0.07          | 0.07          |
| Total               | 100          | 100           | 100           |

<sup>a</sup> Providing the following per kg diet: vitamin A, 4,000 IU; vitamin D3, 400 IU; vitamin E, 16 IU; vitamin K, 1 mg; choline, 200 mg; pantothenic, 6 mg; vitamin B2, 2 mg; folic acid, 0.8 mg; niacin, 10 mg; vitamin B1, 0.6 mg; vitamin B6, 0.6 mg; biotin, 0.08 mg; vitamin B12, 0.01 mg; Cu (as copper sulfate), 15 mg; Fe (as ferrous sulfate), 100 mg; Zn (as zinc oxide), 100 mg; Mn (as manganese sulfate), 20 mg; I (as calcium iodate), 0.3 mg; and Se (as sodium selenite), 0.3 mg

ingredients and nutrient composition of the three experimental diets are summarized in Tables 1 and 2. Pigs were housed in individual stainless steel metabolism cages in a temperature-controlled room (20–22°C) (Kong et al. 2009) and trained for 2 weeks to consume their daily feed allowance of 900 g in two equal meals (0900 and 1700). After this 14-day period of adaptation, catheters were placed into the portal vein, ileal mesenteric vein, and carotid artery, as described below.

### Surgery

Following a 24-h food deprivation, pigs were surgically fitted with chronic catheters in the portal vein, ileal mesenteric vein, and carotid artery, according to the procedures of Yen and Killefer (1987) with two key modifications in the cannulation of the portal vein and ileal mesenteric vein. First, a 2-cm stainless steel tube (2.41-mm O.D. × 1.68-mm I.D.), which was made using adapter luerstus-15 g (VWR International Ltd., Mississauga, ON, Canada), was inserted into the top tip of a portal-vein catheter (Micro-Renathane Tubing, 2.41-mm O.D. × 1.68-mm I.D., Braintree Scientific Inc., NY., USA), as illustrated in

**Table 2** Content of energy and nutrients in experimental diets (as-fed basis)

|                                | Control | Chitosan diet | Pea-hull diet |
|--------------------------------|---------|---------------|---------------|
| Gross energy (MJ/kg)           | 18.99   | 19.02         | 20.00         |
| Nutrient (%)                   |         |               |               |
| Dry matter                     |         |               |               |
| Fat                            | 2.78    | 2.70          | 2.80          |
| Free glucose                   | 0.61    | 0.58          | 0.50          |
| Free sucrose                   | 14.0    | 13.9          | 13.4          |
| Total starch                   | 67.1    | 69.8          | 53.9          |
| Total NSP                      | 1.04    | 1.05          | 6.6           |
| Soluble NSP <sup>a</sup>       | 1.04    | 1.05          | 4.9           |
| Total amino acids <sup>b</sup> | 15.4    | 15.5          | 15.9          |
| Alanine                        | 0.23    | 0.23          | 0.24          |
| Arginine                       | 0.26    | 0.25          | 0.28          |
| Aspartic acid <sup>c</sup>     | 3.90    | 4.00          | 4.12          |
| Cysteine                       | 0.04    | 0.04          | 0.04          |
| Glutamic acid <sup>d</sup>     | 5.15    | 5.12          | 5.23          |
| Glycine                        | 0.15    | 0.15          | 0.16          |
| Histidine                      | 0.27    | 0.26          | 0.27          |
| Isoleucine                     | 0.48    | 0.48          | 0.50          |
| Leucine                        | 0.71    | 0.70          | 0.72          |
| Lysine                         | 0.63    | 0.64          | 0.67          |
| Methionine                     | 0.44    | 0.45          | 0.45          |
| Phenylalanine                  | 0.40    | 0.40          | 0.40          |
| Proline                        | 0.90    | 0.91          | 0.92          |
| Serine                         | 0.41    | 0.40          | 0.42          |
| Threonine                      | 0.32    | 0.30          | 0.34          |
| Tryptophan                     | 0.17    | 0.17          | 0.18          |
| Tyrosine                       | 0.43    | 0.43          | 0.44          |
| Valine                         | 0.52    | 0.52          | 0.53          |

Analyzed values. Except for glycine, all amino acids are L-isomers

<sup>a</sup> NSP, nonstarch polysaccharide

<sup>b</sup> Calculated based on the molecular weights of intact amino acids

<sup>c</sup> Including aspartic acid plus asparagine

<sup>d</sup> Including glutamic acid plus glutamine

Fig. 1. Second, a catheter (Rena-Pulse Tubing, 1.17-mm O.D. × 0.76-mm I.D., Braintree Scientific Inc.) was inserted into the ileal mesenteric vein without any difficulty.

#### Postoperative management

After surgery, pigs were returned to metabolism cages for recovery and received daily intravenous administration of penicillin (6,000 IU/kg body weight) and gentamicin (2 mg/kg body weight) for 5 days. On each day, the catheters were checked for potency, flushed, and filled with heparinized saline solution.



**Fig. 1** An improved catheter for the cannulation of the portal vein in young pigs. A 2-cm stainless steel tube (2.41-mm O.D. × 1.68-mm I.D.), which was made using adapter luerstus-15 g, was inserted into the top tip of a portal-vein catheter (Micro-Renathane Tubing, 2.41-mm O.D. × 1.68-mm I.D.). Use of this catheter greatly facilitated the cannulation of the portal vein in young pigs, thereby substantially reducing surgery time and enhancing post-surgery survival

#### Measurement of the portal-vein blood flow rate (PVBF)

Starting on day 6 post surgery, pigs (approximately 20 kg) were fed 14 days, twice daily at 0900 and 1700, approximately 1,000 g of their respective experimental diets (control, chitosan, or pea hull), depending on the body weight (50 g feed/kg body weight per day). On day 15, after initiation of chitosan or pea-hull supplementation, PVBF was measured using para-aminohippuric acid (PAH) as described by Yen and Killefer (1987) with some modifications. Briefly, PAH solution (1%) was prepared in sterile 0.9% NaCl and filtered through a cellulose acetate membrane (0.5 µm pore size). The solution was adjusted to pH 7.45 and passed through a sterile, disposable 25-mm filter assembly (containing a polysulfonate membrane with 0.2-µm pore size) before its infusion into pigs. At 0730 of the study day, a 1% PAH solution was continuously infused, using a Sp200 Series Syringe Pump (World Precision Instruments, Inc., USA), into the ileal mesenteric vein at a priming rate of 3.82 ml/min for 5 min. After the priming, the infusion rate was changed to 0.79 ml/min for 8.5 h. Blood samples (10 ml) were simultaneously withdrawn from the portal vein and the carotid artery every 60 min using syringes containing ethylenediaminetetraacetic acid. The samples were centrifuged at 4°C at 3,300×g for 10 min and the supernatant fluid (plasma) was obtained (Yin et al. 2009). Concentrations of PAH in plasma were measured using an automated procedure described by Harvey and Brothers (1962).

#### Analysis of AA and glucose in plasma

Amino acids were analyzed using ion-exchange chromatography as we described (Kong et al. 2009; Yin et al. 2009). Authentic standards (Sigma Chemicals, St. Louis, MO, USA) were used to quantify AA in samples. Glucose

was measured using Biochemical Analytical Instrument (Beckman CX4) and a commercial assay kit (Sino-German Beijing Leadman Biotech Ltd., Beijing, China) (Tan et al. 2009b).

### Calculations

PVBF was estimated using the PAH indicator dilution technique (Yen and Killefer, 1987):

$$\text{PVBF} = (C_i \times \text{IR}) / [\text{PAH}_{\text{pv}} \times (1 - \text{HCT}_{\text{pv}}) - \text{PAH}_a \times (1 - \text{HCT}_a)],$$

where PVBF is ml/min,  $C_i$  is PAH concentration in infusion solution (mg/ml), IR is infusion rate (ml/min),  $\text{PAH}_{\text{pv}}$  is PAH concentration (mg/ml) in portal vein plasma,  $\text{PAH}_a$  is PAH concentration (mg/ml) in carotid arterial plasma,  $\text{HCT}_{\text{pv}}$  is hematocrit (%) of portal vein blood, and  $\text{HCT}_a$  is hematocrit (%) of carotid arterial blood.

Portal-vein plasma flow rate (PVPF) was calculated using the following equation:

$$\text{PVPF} = (C_i \times \text{IR}) / [\text{PAH}_{\text{pv}} \times (1 - \text{HCT}_{\text{pv}})],$$

where PVPF is ml/min,  $C_i$  is PAH concentration in infusion solution (mg/ml), IR is infusion rate (ml/min),  $\text{PAH}_{\text{pv}}$  is PAH concentration (mg/ml) in portal vein plasma, and  $\text{HCT}_{\text{pv}}$  is hematocrit (%) of portal vein blood.

Rates of PDV oxygen consumption and the net appearance of dietary glucose in the portal vein were calculated based on PVBF and concentration differences between portal-vein and carotid-arterial blood. Rates of net appearance of dietary AA in the portal vein were calculated based on PVPF and concentration differences between the portal-vein and carotid-arterial plasma (Li et al. 2008).

### Statistical analysis

Data were subjected to one-way analysis of variance using the General Linear Model procedure of SAS (SAS Inst. Inc., Cary, NC, USA). Differences among treatment mean were determined by the Tukey multiple comparison test.  $P$  values  $\leq 0.05$  were taken to indicate significance.

## Results

### General aspects of the surgery

The surgical procedures for placing chronic catheters into the portal vein, ileal mesenteric vein, and carotid artery of young pigs lasted approximately 2.5 h per animal, which was approximately 1 h less than that reported by Yen and Killefer (1987). After the surgery, all of the pigs remained

healthy and consumed their daily feed allowance. In addition, all three groups of pigs grew normally throughout the 14-day study and their weight gain did not differ ( $P > 0.05$ ). A postmortem examination at 2 months after the surgery showed that (1) the stainless-steel tube had no rust or damage; and (2) all catheters were still fitted in the desired position and functioned well.

### Portal-vein blood flow and oxygen consumption by the PDV

PVBF did not differ ( $P > 0.05$ ) among pigs fed the control, chitosan, and pea-hull diets (Table 2). The average values were 31.5 ml/kg body weight per min. However, rates of PDV oxygen consumption (expressed per kg body weight and per 100 g feed intake) differed ( $P < 0.05$ ) markedly among the three groups of pigs (Table 3). Specifically, compared with the control group, dietary chitosan decreased ( $P < 0.05$ ) PDV oxygen consumption by 34%, whereas dietary pea-hull increased ( $P < 0.05$ ) PDV oxygen consumption by 31%. The rate of oxygen consumption by the PDV of pea hull-supplemented pigs doubled the value for chitosan-supplemented pigs.

### Proportions of dietary AA and glucose appearing in the portal vein after feeding

Concentrations of AA in the portal vein and carotid-arterial were similar to those we previously reported for young pigs (Li et al. 2008). Rates of the entry of dietary total measured AA and glucose into the portal vein after feeding are summarized in Table 4. Approximately, 69 and 82% of dietary total measured AA and glucose appeared in the portal vein of control pigs, respectively, during an 8-h period after feeding. Dietary supplementation with chitosan increased ( $P < 0.05$ ) the entry of dietary AA into the portal vein by 28%, compared with the control group. In contrast, an opposite result was obtained for pea-hull supplementation. Rates of dietary glucose entering the portal vein were 16% lower ( $P < 0.05$ ) in the pea-hull group than in the control group. There were no differences ( $P > 0.05$ ) in the appearance of dietary glucose in the portal vein between the control and chitosan-supplemented pigs.

Dietary supplementation with chitosan increased ( $P < 0.05$ ) rates of the entry of dietary alanine, arginine, glutamate, glycine, lysine, and methionine into the portal vein, compared with control and pea hull-supplemented pigs (Table 4). In contrast, rates of the entry of dietary alanine, arginine, glutamate, glycine, lysine, and threonine into the portal vein were lower ( $P < 0.05$ ) in pea hull-supplemented pigs than in the control group. Supplementation with either chitosan or pea hull had no effect ( $P > 0.05$ ) on the appearance of dietary aspartate, BCAA,

**Table 3** Portal-vein blood flow rate and oxygen consumption by the portal-drained viscera of young pigs during 8 h after feeding

| Variable                                                    | Control diet     | Chitosan diet    | Pea-hull diet    | Pooled SEM | <i>P</i> value |
|-------------------------------------------------------------|------------------|------------------|------------------|------------|----------------|
| Portal-vein blood flow rate (ml/kg body wt per min)         | 30.8             | 32.3             | 31.2             | 1.6        | 0.330          |
| O <sub>2</sub> consumption (ml/kg BW per 100 g feed intake) | 155 <sup>a</sup> | 102 <sup>b</sup> | 203 <sup>c</sup> | 11         | 0.047          |

Pigs were fed a cornstarch and casein-based diet (Control) supplemented with 0, 12% pea hull (containing 95% of FNSP), or 100 mg chitosan/kg diet (COS)

Values are mean  $\pm$  SEM,  $n = 5$

<sup>a-c</sup> Mean in a row with different superscript letters differ ( $P < 0.05$ )

**Table 4** Proportions (%) of dietary amino acids and glucose appearing in the portal vein of young pigs during 8 h after feeding

| Nutrients         | Control diet     | Chitosan diet    | Pea-hull diet     | SEM | <i>P</i> value |
|-------------------|------------------|------------------|-------------------|-----|----------------|
| Alanine           | 351 <sup>b</sup> | 457 <sup>a</sup> | 271 <sup>c</sup>  | 15  | 0.048          |
| Arginine          | 74 <sup>b</sup>  | 86 <sup>a</sup>  | 53 <sup>c</sup>   | 3.6 | 0.046          |
| Aspartate         | 9.8              | 12               | 7.2               | 4.5 | 0.245          |
| Glutamate         | 8.6 <sup>b</sup> | 33 <sup>a</sup>  | −3.9 <sup>c</sup> | 5.9 | 0.044          |
| Glycine           | 81 <sup>b</sup>  | 113 <sup>a</sup> | 50 <sup>c</sup>   | 5.4 | 0.041          |
| Histidine         | 72               | 87               | 61                | 5.3 | 0.493          |
| Isoleucine        | 52               | 60               | 46                | 6.2 | 0.589          |
| Leucine           | 49               | 55               | 40                | 5.9 | 0.647          |
| Lysine            | 54 <sup>b</sup>  | 71 <sup>a</sup>  | 38 <sup>c</sup>   | 6.5 | 0.049          |
| Methionine        | 50 <sup>b</sup>  | 71 <sup>a</sup>  | 46 <sup>b</sup>   | 3.8 | 0.044          |
| Phenylalanine     | 30               | 34               | 32                | 2.4 | 0.789          |
| Serine            | 46               | 51               | 41                | 4.0 | 0.845          |
| Threonine         | 51 <sup>a</sup>  | 60 <sup>a</sup>  | 30 <sup>b</sup>   | 5.4 | 0.046          |
| Tyrosine          | 57               | 60               | 50                | 4.6 | 0.784          |
| Valine            | 54               | 61               | 46                | 5.8 | 0.744          |
| Total measured AA | 69 <sup>b</sup>  | 88 <sup>a</sup>  | 54 <sup>c</sup>   | 2.1 | 0.023          |
| Glucose           | 82 <sup>a</sup>  | 85 <sup>a</sup>  | 70 <sup>b</sup>   | 3.2 | 0.045          |

Values are mean  $\pm$  SEM,  $n = 5$ . Except for glycine, all amino acids are L-isomers

<sup>a-c</sup> Mean in a row with different superscript letters differ ( $P < 0.05$ )

histidine, phenylalanine, serine or tyrosine in the portal vein. In the three groups of pigs, among all measured AA, only alanine exhibited a value greater than >100% for appearance in the portal vein relative to dietary intake (Table 4).

## Discussion

In vivo animal models are valuable to quantify AA metabolism by the PDV in the presence of all physiological substances (Burrin and Reeds 1997; Huntington 1982). The technique described here for the cannulation of the portal vein, ileal mesenteric vein, and carotid artery substantially reduced surgery time and mortality in young pigs. The improvement was mainly due to: (1) use of a new catheter (Fig. 1) that could be easily inserted into the portal vein; and (2) the ileal mesenteric-vein catheter (Rena-Pulse Tubing, Braintree Scientific Inc.) used in the present experiment being harder than the catheter (Micro-Renathane Tubing, Braintree Scientific Inc.) used in previous studies (Yen and Killefer 1987). Values for PVBF (ml/kg

body weight per min) and PDV oxygen consumption (ml/kg body weight per 100 g feed intake) obtained from the present study (Table 3) are very similar to those reported for growing pigs by Rerat et al. (1984) and Yen and Killefer (1987). These results indicate that our technique is valid for studying AA metabolism in the PDV of pigs. The usefulness of the method for nutrition research is further supported by the findings that dietary supplementation with chitosan or pea hull differentially affected PDV oxygen consumption (Table 3), as well as the entry of dietary AA and glucose into the portal vein of young pigs (Table 4).

The small intestine is known to extensively utilize both glutamate and aspartate for ATP production (Burrin et al. 2000). This is also true for young pigs fed a casein- and cornstarch-based diet supplemented with 3.3% glutamate and 3.3% aspartate (Table 4). The small-intestinal mucosa also degrades glutamine (Wu et al. 1995), arginine (Wu et al. 1996), proline (Wu 1997), and BCAA (Chen et al. 2007, 2009), such that 30–50% of these dietary AA do not enter the portal circulation (Stoll et al. 1998; Wu 1998). It is now clear that bacteria in the lumen of the pig small intestine actively utilize nutritionally essential and

nonessential AA for both oxidation and protein synthesis (Dai et al. 2010), which may explain the previous findings that large amounts of dietary essential AA (including BCAA, histidine, lysine, methionine, phenylalanine, and threonine) do not appear in the portal circulation (Stoll et al. 1998; Stoll and Burrin 2006). Because dietary AA are major fuels for the small-intestinal mucosa and essential precursors for the intestinal synthesis of proteins, glutathione, polyamines, nitric oxide, purines, and pyrimidines, intestinal AA metabolism is obligatory for maintaining intestinal mucosal mass, function, and integrity (Wu et al. 2005). Based on (a) PDV oxygen consumption (2.16 mol O<sub>2</sub>/day) for a 25-kg pig fed daily 1.25-kg diet (Table 2); (b) PDV utilization of dietary glutamate (0.38 mol/day) and aspartate (0.34 mol/day) (Tables 1, 4) with 52% of them being oxidized by mucosal cells (Stoll et al. 1999; Wu 1998); and (c) the requirements of 4.5 and 3 mol O<sub>2</sub> for complete oxidation of 1 mol glutamate and 1 mol aspartate, respectively (Wu 2009), we estimated that oxidation of dietary glutamate and aspartate accounted for 41 and 25% of PDV oxygen consumption, respectively, under the experimental conditions of the present study.

A new finding of this work is that rates of the appearance of lysine and threonine in the portal vein of young pigs were substantially lower than those previously reported for the efficiency of these two AA in the diet for whole body protein deposition (Libao 2002), even though it was assumed that as much as 15% of body protein retention occurred in the PVD (Stoll et al. 1998; Stoll and Burrin 2006). It is likely that the net appearance of free lysine and threonine in the portal blood plasma underestimates their supply from the diet to peripheral tissues. This may be due to the transport of both AA via blood cells (Le Floc'h et al. 1999). It is also possible that some of the dietary lysine and threonine may appear in the portal vein in the form of small peptides and, therefore, they were not detected as free AA in the portal-vein plasma. Transport of peptides may be an important mechanism for interorgan metabolism of some AA.

Another novel observation from the present study is that dietary pea-hull reduced, but dietary chitosan increased, the net absorption of many dietary AA into the portal vein (Table 4). The underlying mechanisms may involve primarily changes in AA metabolism by the gut microflora due to alterations in the species of bacteria as well as their numbers and activities (Bergen and Wu 2009; Metzler and Mosenthin 2008; Mosenthin et al. 1994; Schulze et al. 1995; Souffrant et al. 1993; Wang et al. 2009b; Dai et al. 2010). Nonetheless, an increase in the circulating levels of AA could promote protein synthesis in young pigs (Davis et al. 1998, 2000; Suryawan et al. 2009; Yao et al. 2008) via mTOR and possibly additional signaling pathways (Li et al. 2009; Palii et al. 2009; Rhoads and Wu 2009). This is consistent with the previous observation that dietary

supplementation with 100 mg/kg of chitosan enhanced body-weight gain and feed efficiency in 15- to 45-day-old piglets (Tang et al. 2005) and 0- to 44-day-old broilers (Huang et al. 2005, 2007).

In summary, we described an improved technique for successfully implanting chronic catheters into the portal vein, ileal mesenteric vein, and carotid artery of young pigs. Using this method, we found that dietary chitosan decreased, but dietary pea-hull increased, PDV oxygen consumption as well as the net absorption of dietary AA and glucose into the portal vein of young pigs. These results indicate that chitosan is an effective prebiotic for enhancing the efficiency of utilization of dietary protein and AA in swine production.

**Acknowledgments** This research was supported by grants from National 863 project (2008AA10Z316), Chinese Academy of Sciences and Knowledge Innovation Project (KSCX2-YW-N-051), National Natural Science Foundation of China (30901040, 30901041, 3092801, 30828025, and 30771558), Guangdong Project (2009B091300079), K.-C. Wong Education Foundation of Hong Kong, NSFC (30528006, 30671517, 30700581, 30771558, 0371038, and 30928018), Fund of Agricultural Science and Technology outcome application (2006GB24910468), Foundation of Hunan Province (2007FJ1003), and Texas AgriLife Research (H-8200).

## References

- Bergen WG, Wu G (2009) Intestinal nitrogen recycling and utilization in health and disease. *J Nutr* 139:821–825
- Blachier F, Mariotti F, Huneau JF et al (2007) Effects of amino acid-derived luminal metabolites on the colonic epithelium and physiopathological consequences. *Amino Acids* 33:547–562
- Blachier F, Boutry C, Bos C et al (2009) Metabolism and functions of L-glutamate in the Epithelial cells of the small and large intestines. *Am J Clin Nutr* 90:814S–821S
- Burrin DG, Reeds PJ (1997) Alternative fuels in the gastrointestinal tract. *Curr Opin Gastroenterol* 13:165–170
- Burrin DG, Stoll B, van Goudoever JB (2000) Nutrient requirements for intestinal growth and metabolism in the developing pig. In: Lindberg JE, Ogle B et al (eds) *Digestive Physiology of pig*. CAB International, Wallingford, UK, pp 75–88
- Chen LX, Yin YL, Jobgen WS et al (2007) In vitro oxidation of essential amino acids by jejunal mucosal cells of growing pigs. *Livest Sci* 109:19–23
- Chen LX, Li P, Wang JJ, Li XL et al (2009) Catabolism of nutritionally essential amino acids in developing porcine enterocytes. *Amino Acids* 37:143–152
- Dai ZL, Zhang J, Wu G et al (2010) Utilization of amino acids by bacteria from the pig small intestine. *Amino Acids*. doi: 10.1007/s00726-010-0556-9
- Davis TA, Burrin DG, Fiorotto ML et al (1998) Role of insulin and amino acids in the regulation of protein synthesis 438 in the neonate. *J Nutr* 128:347S–350S
- Davis TA, Nguyen HV, Suryawan A et al (2000) Developmental changes in the feeding-induced stimulation of translation initiation in muscle of neonatal pigs. *Am J Physiol Endocrinol Metab* 279:E1226–E1234
- Deng D, Yin YL, Chu WY et al (2009) Impaired translation initiation activation and reduced protein synthesis in weaned piglets fed a low-protein diet. *J Nutr Biochem* 20:544–552

- Harvey RB, Brothers AJ (1962) Renal extraction of para-aminohippurate and creatinine measured by continuous *in vivo* sampling of arterial and renal-vein blood. *Ann NY Acad Sci* 102:46–53
- Huang RL, Yin YL, Wu GY et al (2005) Effect of dietary oligochitosan supplementation on ileal digestibility of nutrients and performance in Broilers. *Poult Sci* 84:1383–1388
- Huang RL, Yin YL, Li MX et al (2007) Dietary oligochitosan supplementation enhances immune status of broilers. *J Sci Food Agric* 87:153–159
- Huntington GB (1982) Portal blood flow and net absorption of ammonia-nitrogen, urea-nitrogen and glucose in nonlactating Holstein cow. *J Dairy Sci* 65:1155–1162
- Kong XF, Yin YL, He QH et al (2009) Dietary supplementation with Chinese herbal powder enhances ileal digestibilities and serum concentrations of amino acids in young pigs. *Amino Acids* 37:573–582
- Le Floc'h N, Meziere N, Seve B (1999) Whole blood and plasma amino acid transfers across the portal drained viscera and liver of the pig. *Reprod Nutr Dev* 39:433–442
- Li TJ, Dai QZ, Yin YL et al (2008) Dietary starch sources affect net portal appearance of amino acids and glucose in growing pigs. *Animal* 2:723–729
- Li XL, Bazer FW, Gao HJ et al (2009) Amino acids and gaseous signaling. *Amino Acids* 37:65–78
- Libao AJ (2002) Utilization of amino acids for protein deposition in the growing pig: effect of dietary protein source. MS thesis, University of Guelph, Guelph, Canada
- Ligthart-Melis GC, van de Poll MCG, Boelens PG et al (2008) Glutamine is an important precursor for *de novo* synthesis of arginine in humans. *Am J Clin Nutr* 87:1282–1289
- Metzler BU, Mosenthin R (2008) A review of interactions between dietary fiber and the gastrointestinal microbiota and their consequences on intestinal phosphorus metabolism in growing pigs. *Asian Australas J Anim Sci* 21:603–615
- Mosenthin R, Sauer WC, Ahrens F (1994) Dietary pectin's effect on ileal and fecal amino acid digestibility and exocrine pancreatic secretions in growing pigs. *J Nutr* 124:1222–1229
- Palii SS, Kays CE, Deval C et al (2009) Specificity of amino acid regulated gene expression: analysis of gene subjected to either complete or single amino acid deprivation. *Amino Acids* 37:79–88
- Rerat A, Vaissade P, Pvaugelade P (1984) Absorption kinetics of some carbohydrates in conscious pigs. *Br J Nutr* 51:505–515
- Rhoads JM, Wu G (2009) Glutamine, arginine, and leucine signaling in the intestine. *Amino Acids* 37:111–122
- Schulze H, van Leeuwen P, Verstegen MWA et al (1995) Dietary level and source of neutral detergent fiber and ileal endogenous nitrogen flow in pigs. *J Anim Sci* 73:441–448
- Souffrant WB, Rerat A, Laplace JP et al (1993) Exogenous and endogenous contribution to nitrogen fluxes in the digestive tract of pigs fed a casein diet. III. Recycling of endogenous nitrogen. *Reprod Nutr Dev* 33:373–382
- Stoll B, Burrin DG (2006) Measuring splanchnic amino acid metabolism *in vivo* using stable isotopic tracers. *J Anim Sci* 84:E60–E72
- Stoll B, Burrin DG, Yu H et al (1998) Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. *J Nutr* 128:606–614
- Stoll B, Burrin DG, Henry J, Yu H, Jahoor F, Reeds PJ (1999) Substrate oxidation by the portal drained viscera of fed piglets. *Am J Physiol* 277:E168–E175
- Suryawan A, O'Connor PMJ, Bush JA et al (2009) Differential regulation of protein synthesis by amino acids and insulin in peripheral and visceral tissues of neonatal pigs. *Amino Acids* 37:97–104
- Tan BE, Li XG, Kong XF et al (2009a) Dietary L-arginine supplementation enhances the immune status in early-weaned piglets. *Amino Acids* 37:323–331
- Tan BE, Yin YL, Liu ZQ et al (2009b) Dietary L-arginine supplementation increases muscle gain and reduces body fat mass in growing-finishing pigs. *Amino Acids* 37:169–175
- Tang ZR, Yin YL, Nyachoti CM et al (2005) Effect of dietary supplementation of chitosan and galacto-mannan-oligosaccharides on serum parameters and the insulin-like growth factor-I mRNA expression in early-weaned piglets. *Domest Anim Endocrinol* 28:430–441
- van de Poll MCG, Siroen MPC, van Leeuwen PAM et al (2007) Interorgan amino acid exchange in humans: consequences for arginine and citrulline metabolism. *Am J Clin Nutr* 85:167–172
- Wang WW, Qiao SY, Li DF (2009a) Amino acids and gut function. *Amino Acids* 37:105–110
- Wang X, Ou D, Yin J et al (2009b) Proteomic analysis reveals altered expression of proteins related to glutathione metabolism and apoptosis in the small intestine of zinc oxide-supplemented piglets. *Amino Acids* 37:209–218
- Wu G (1997) Synthesis of citrulline and arginine from proline in enterocytes of postnatal pigs. *Am J Physiol Gastrointest Liver Physiol* 272:G1382–G1390
- Wu G (1998) Intestinal mucosal amino acid catabolism. *J Nutr* 128:1249–1252
- Wu G (2009) Amino acids: metabolism, functions, and nutrition. *Amino Acids* 37:1–17
- Wu G, Borbolla AG, Knabe DA (1994) The uptake of glutamine and release of arginine, citrulline and proline by the small intestine of developing pigs. *J Nutr* 124:2437–2444
- Wu G, Knabe DA, Yan W et al (1995) Glutamine and glucose metabolism in enterocytes of the neonatal pig. *Am J Physiol Regul Integr Comp Physiol* 268:R334–R342
- Wu G, Knabe DA, Flynn NE et al (1996) Arginine degradation in developing porcine enterocytes. *Am J Physiol Gastrointest Liver Physiol* 271:G913–G919
- Wu G, Knabe DA, Flynn NE (2005) Amino acid metabolism in the small intestine: biochemical bases and nutritional significance. In: Burrin DG, Mersmann HJ (eds) *Biology of metabolism of growing animals*. Elsevier, New York, pp 107–126
- Yao K, Yin YL, Chu W et al (2008) Dietary arginine supplementation increases mTOR signaling activity in skeletal muscle of neonatal pigs. *J Nutr* 138:867–872
- Yen JT, Killefer J (1987) A method for chronically quantifying net absorption of nutrients and gut metabolites into hepatic portal vein in conscious swine. *J Anim Sci* 64:923–934
- Yin FG, Liu YL, Yin YL et al (2009) Dietary supplementation with Astragalus polysaccharide enhances ileal digestibilities and serum concentrations of amino acids in early weaned piglets. *Amino Acids* 37:263–270