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SHORT COMMUNICATION

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

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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,

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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 ± 1 kg) were randomly allocated into one of three dietary groups ($n = 5$/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 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.

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[a]	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

[a] 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

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[a]	1.04	1.05	4.9
Total amino acids[b]	15.4	15.5	15.9
Alanine	0.23	0.23	0.24
Arginine	0.26	0.25	0.28
Aspartic acid[c]	3.90	4.00	4.12
Cysteine	0.04	0.04	0.04
Glutamic acid[d]	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

[a] NSP, nonstarch polysaccharide

[b] Calculated based on the molecular weights of intact amino acids

[c] Including aspartatic acid plus asparagine

[d] 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.

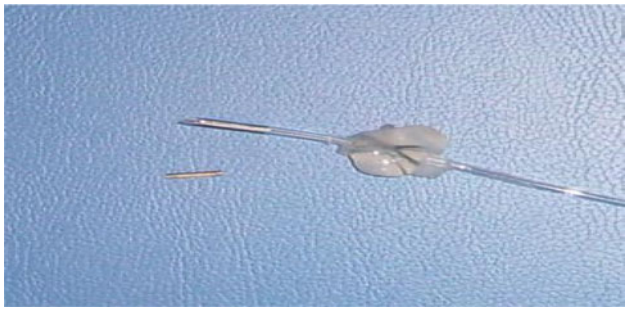

Fig. 1 An improved catheter for the cannulation of the portal vein in young pigs. A 2-cm stainless steel tube (2.41-mm O.D. × 1.68-mm I.D.), which was made using adapter luerstus-15 g, was inserted into the top tip of a portal-vein catheter (Micro-Renathane Tubing, 2.41-mm O.D. × 1.68-mm I.D.). Use of 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):

$$\mathrm{PVBF} = (C_\mathrm{i} \times \mathrm{IR})/\left[\mathrm{PAH_{pv}} \times \left(1 - \mathrm{HCT_{pv}}\right) - \mathrm{PAH_a} \times (1 - \mathrm{HCT_a})\right],$$

where PVBF is ml/min, C_i is PAH concentration in infusion solution (mg/ml), IR is infusion rate (ml/min), $\mathrm{PAH_{pv}}$ is PAH concentration (mg/ml) in portal vein plasma, $\mathrm{PAH_a}$ is PAH concentration (mg/ml) in carotid arterial plasma, $\mathrm{HCT_{pv}}$ is hematocrit (%) of portal vein blood, and $\mathrm{HCT_a}$ is hematocrit (%) of carotid arterial blood.

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

$$\mathrm{PVPF} = (C_\mathrm{i} \times \mathrm{IR})/\left[\mathrm{PAH_{pv}} \times \left(1 - \mathrm{HCT_{pv}}\right)\right],$$

where PVPF is ml/min, C_i is PAH concentration in infusion solution (mg/ml), IR is infusion rate (ml/min), $\mathrm{PAH_{pv}}$ is PAH concentration (mg/ml) in portal vein plasma, and $\mathrm{HCT_{pv}}$ 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 ≤ 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	*P* value
Portal-vein blood flow rate (ml/kg body wt per min)	30.8	32.3	31.2	1.6	0.330
O_2 consumption (ml/kg BW per 100 g feed intake)	155[a]	102[b]	203[c]	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 ± SEM, $n = 5$

[a–c] 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	*P* value
Alanine	351[b]	457[a]	271[c]	15	0.048
Arginine	74[b]	86[a]	53[c]	3.6	0.046
Aspartate	9.8	12	7.2	4.5	0.245
Glutamate	8.6[b]	33[a]	−3.9[c]	5.9	0.044
Glycine	81[b]	113[a]	50[c]	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[b]	71[a]	38[c]	6.5	0.049
Methionine	50[b]	71[a]	46[b]	3.8	0.044
Phenylalanine	30	34	32	2.4	0.789
Serine	46	51	41	4.0	0.845
Threonine	51[a]	60[a]	30[b]	5.4	0.046
Tyrosine	57	60	50	4.6	0.784
Valine	54	61	46	5.8	0.744
Total measured AA	69[b]	88[a]	54[c]	2.1	0.023
Glucose	82[a]	85[a]	70[b]	3.2	0.045

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

[a–c] 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 throenine) 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_2/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_2 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.

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