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ORIGINAL ARTICLE

Effect of graded levels of dietary betaine on ileal and total tract nutrient digestibilities and intestinal bacterial metabolites in piglets*

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Summary

The study was conducted to investigate the effects of graded dietary inclusion levels of betaine on ileal and total tract nutrient digestibilities and intestinal bacterial metabolites in piglets. A total of eight barrows with an average initial body weight of 7.9 kg were randomly allocated to one of the four assay diets with two pigs per treatment in four repeated measurement periods. The assay diets included a basal diet based on wheat, barley and soybean meal alone, or supplemented with a liquid betaine product at dietary levels of 1.5, 3.0, or 6.0 g betaine per kilogram diet (as-fed). Ileal digestibilities of dry matter and neutral detergent fibre increased both quadratically and linearly, and ileal digestibility of glycine increased linearly as dietary betaine level increased ($p < 0.05$). Furthermore, total tract digestibility of crude protein increased quadratically ($p < 0.05$) and total tract digestibilities of most amino acids tended to increase quadratically ($p = 0.06$ to $p = 0.11$) with increasing dietary betaine level. Moreover, there were linear increases in the concentrations of most bacterial metabolites which were significant $p < 0.05$ for ileal D-lactic acid and for faecal diaminopimelic acid. The results demonstrate that dietary betaine supplementation stimulates bacterial fermentation of fibre in the small intestine and bacterial degradation of crude protein in the large intestine.

Introduction

During the last decade, considerable research has been performed to study the efficacy of betaine to improve growth performance in pigs (Yu et al., 2004; Huang et al., 2006; Fernandez-Figares et al., 2008). Betaine is the trimethyl derivative of the amino acid glycine and acts as osmoprotectant in plants (Xing and Rajashekar, 2001), bacteria (Pichereau et al., 1999), marine organisms (Clarke et al., 1994), and numerous vertebrate tissues (Law and Burg, 1991). The osmoprotective properties of betaine likely influence nutrient digestibility by supporting growth and survival of intestinal cells as well as intestinal microbes (Eklund et al., 2005). Furthermore, there is an increasing evidence that dietary betaine supplementation alters the composition of the bacterial community in the gastrointestinal tract of piglets (Eklund et al., 2006a; b) and poultry (Kettunen et al., 1999). Because both intestinal cells and intestinal bacteria have to cope with varying osmotic conditions along the digestive tract (Mongin, 1976), dietary betaine supplementation may aid intestinal cells as well as intestinal bacteria to compensate for this variation (Eklund et al., 2006b). Only few studies have been conducted so far with the focus on potential effects of betaine on nutrient digestibility in pigs, although there is an evidence that dietary betaine supplementation may increase the digestibility of neutral detergent fibre, acid detergent fibre, amino acids and minerals along the gastrointestinal tract of piglets (Eklund et al., 2006a; b). Among studies, there exist considerable variations in the level of betaine supplementation, and betaine effects among studies were of different magnitude (Xu and Yu, 2000; Eklund et al., 2006a; b; Fernandez-Figares et al., 2008). Moreover, the effect of betaine on ileal and total tract nutrient digestibilities and on bacterial degradation of nutrients as influenced by the level of betaine in the diet has not yet been determined in piglets. Therefore, the objective of the study was to investigate the effect of graded levels of dietary betaine supplementation on ileal and total tract nutrient digestibilities and intestinal bacterial metabolites in piglets.

Materials and methods

Animals and housing

A total of 8 five-week-old barrows (German Landrace × Piétrain) were obtained from the University of Hohenheim research unit. The average initial and final body weight of the pigs was 7.9 ± 0.3 and 27.3 ± 1.7 kg respectively. The pigs were individually housed in stainless-steel metabolic crates. During the adaptation period until 3 to 5 days after surgery, all the pigs were fed increasing amounts of the basal diet. The temperature in the research unit, which was equipped with an automatic temperature control system, was set at 25 °C. Each crate was equipped with an infrared heating lamp and a low-pressure drinking nipple.

Surgical procedure

The piglets were surgically fitted with simple T-cannulas at the distal ileum on day 7 and 9 after weaning according to the principles described by Li et al. (1993). The cannulas were prepared from a plastisol solution (Techniplast, FH and Sons, Toronto, Canada) according to procedures as outlined by Li et al. (1993). The internal diameter of the barrel of the cannulas was 12 mm, the total diameter was 16 mm, the length of the barrel was 50 mm and each of the two flanges was 35 mm in length. The washer had 50 mm in diameter and a short barrel of 15 mm of length. Natural rubber plugs were used as stoppers. The research protocol was approved by the German Ethical Commission for Animal Welfare. Care of the animals used in this experiment was in accordance with the guidelines issued by the German regulation for care and treatments of animals (Lorz and Metzger, 1999).

Dietary treatments and experimental design

The basal diet consisted of wheat, barley, soybean meal, cornstarch, a mineral and vitamin premix and titanium dioxide as a digestibility marker (Table 1). All ingredients were ground through a 2-mm mesh screen before being included in the diet. The diet was formulated to meet or to exceed the NRC (1998) nutrient requirements for piglets from 10 to 20 kg of body weight. Graded levels of a liquid betaine product, which contained 563 g dry matter (analysed), 96 g crude ash (analysed), 79 g nitrogen (analysed), and 250 g betaine (manufacturer's specification) per kilogram liquid product, were supplemented to the basal diet at the expense of cornstarch. The basal diet was defined as control treatment. The three assay diets were formulated to contain multiple levels of 1.5, 3.0 and 6.0 g supplemental betaine per kilogram diet (as-fed). The experimental animals were assigned at random to the 4 dietary treatments. The experiment was conducted as four repeated measurement periods with two pigs per treatment ($n = 2$, observations = 8 per treatment). The experimental diets were fed in a mash form, mixed with water (1/1 w/v), twice daily at 07:00 and 19:00 hours. Within each measurement period, the daily feed allowance was set at 45 g/kg (as-fed) of individual body weight. The pigs had free access to water.

Table 1 Formulation of the basal diet (g/kg as-fed)

Ingredient	
Barley	376
Wheat	311
Soybean meal	180
Cornstarch	68
Soybean oil	20
Mineral and vitamin premix*	40
Titanium dioxide	5

*The mineral and vitamin premix supplied the following per kilogram diet: 14 400 IU vitamin A; 1 600 IU vitamin D3; 120 mg vitamin C; 160 mg vitamin E; 2.4 mg vitamin K3; 2.56 mg vitamin B1; 5.76 mg vitamin B2; 3.2 mg vitamin B6; 38.4 μg vitamin B12; 1.44 mg folic acid; 32 mg nicotinic acid; 24 mg pantothenic acid; 240 mg biotin; 250 mg choline chloride; 6.2 g Ca; 2.4 g P; 1.4 g Na; 1.2 g Mg; 140 mg Cu; 100 mg Zn; 75 mg Mn; 120 mg Fe; 0.45 mg Se; 1.8 mg Co; 3 mg I; 4 g L-lysine HCl; 1.2 g DL-methionine; 1.8 g L-threonine; 0.32 g L-tryptophan.

Sample collection

During each of the four experimental periods, the pigs were allowed to adapt to their daily feed allowance for 5 days before faeces were collected. The collection of faeces was initiated at 07:00 hours on day 6 and continued for 72 consecutive hours using adhesive collection bags attached to the anus. Faeces were collected twice daily. Sub-samples of fresh faeces for the analysis of lactic acids and short-chain fatty acids (SCFA) were taken and stored at -30 °C until analysis. The remaining faecal samples were pooled, mixed within animal and period, and freeze-dried thereafter. Ileal digesta were collected for 2 × 12 h, from 07:00 to 19:00 hours on day 9 and from 19:00 to 07:00 hours on day 10 of each experimental period. The collection procedure for ileal digesta was adapted from Li et al. (1993) using plastic tubing attached to the barrel of the cannula by elastic bands. The plastic tubing was changed at least every 20 min. During digesta collection, 2 ml 2.5 M formic acid was added to the plastic tubing to minimize further bacterial fermentation in digesta, except for the samples for the determination of lactic acid and SCFA concentrations. Samples for lactic acid and SCFA were collected for 20 min at 2 h-intervals during each digesta collection period, and stored at -30 °C until analysis. The remaining ileal digesta samples were pooled, mixed within animal and period, and freeze-dried thereafter. Samples of diets, ileal digesta and faeces were milled through a 1.0-mm mesh screen prior to analyses.

Chemical analyses

Determination of dry matter (DM), crude ash (CA), crude protein (CP), ether extracts (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF) and amino acids (AA) was performed as outlined by Naumann and Bassler (1997). Amino acid analyses including diaminopimelic acid (DAP) and ornithine (Orn) measurements were performed by means of ion exchange chromatography (Amino Acid Analyzer LC 300, Eppendorf Biotronic) using post-column derivatization with ninhydrin. For the determination of sulphur-containing AA, the samples were oxidised with performic acid prior to hydrolysis. The D- and L-lactic acid concentrations were determined by means of a photometric test kit (Boehringer, No. 1 112 821). Short-chain fatty acid concentrations were measured by gas chromatography using 4-methyl-iso-valerianic acid as internal standard. Samples for SCFA analyses were prepared according to the principles described by Zijlstra et al. (1977). The titanium dioxide contents in feed, ileal digesta and faecal samples were determined photometrically according to the procedures described by Brandt and Allam (1987).

Statistical analyses

Initially, the following linear model for selecting a repeated correlation structure was considered: $y_{ijk} = \mu + \beta_j + \delta_i + \beta_i \times \delta_i + e_{ijk}$, where y_{ijk} = jth measurement on kth animal in ith betaine level, μ = general term (fixed), β_j = effect of jth period (fixed), δ_i = effect of ith betaine level (fixed), e_{ijk} = error associated with y_{ijk} (random). The errors e_{ijk} of repeated measurements on the same subject (animal within betaine level group) are assumed to be serially correlated. Different serial correlation structures were fitted by the REML method as implemented in the MIXED procedure of SAS (2003) and the best structure according to the Akaike Information Criterion was selected. The following models were considered for e_{ijk}: independent, independent + animal effect (compound symmetry), AR(1) and AR(1) + animal effect. Using the selected correlation structure, treatment effects were modelled by linear and quadratic regression on betaine levels. The significance level for all Wald-type F-tests was set at $\alpha = 0.05$.

Results and discussion

General observations

All animals seemed healthy throughout the experiment and readily consumed their feed allowances. However, for one animal of the treatment with 3.0 g supplemental betaine per kilogram diet, a 6.7% lower ileal DM digestibility compared with the average of all treatments was observed in period 1. Therefore, observations at the ileal level for this animal in this period were removed from the data set. In total, eight (n = 2, periods = 4) observations for each of the four treatments, both at the ileal and the faecal level, were included in the model, except for the treatment with 3.0 supplemental betaine per kilogram diet with seven (n = 2, periods = 4) observations at the ileal level. The analysed chemical composition of the basal diets is presented in Table 2. The nutrient concentrations in the assay diets were in good agreement with those calculated from the single ingredients.

Ileal and total tract digestibility of dry matter, neutral detergent fibre and acid detergent fibre

The ileal digestibilities of DM and NDF increased, both quadratically ($p < 0.05$) and linearly ($p < 0.05$) as dietary betaine level increased (Table 3). The highest increase in ileal DM and NDF digestibilities was obtained when 3.0 g betaine per kilogram assay diet was supplemented, amounting to 1.9% and 11.2% units increase for DM and NDF, respectively, compared with the control treatment. However, a further increase in dietary betaine level from 3.0 to 6.0 g betaine per kilogram assay diet had no additional effect on ileal digestibilities of DM or NDF. There was no effect of betaine supplementation on ileal digestibility of ADF, probably due to the large variation in digestibility coefficients among pigs (Fernandez and Jorgensen, 1986). Furthermore, betaine supplementation to the assay diets did not affect ($p > 0.05$) total tract digestibilities of DM, NDF or ADF (Table 4).

Table 2 Analysed nutrient composition of the basal diet (g/kg dry matter)*

Nutrient composition	
Dry matter	887.0
Crude protein	176.0
Ether extracts	46.0
Crude ash	62.0
Neutral detergent fibre	125.0
Acid detergent fibre	47.0
Calcium	8.0
Phosphorus	5.7
Metabolizable Energy (MJ/kg)†	14.8
Indispensable AA	
Arginine	10.5
Histidine	4.1
Isoleucine	6.4
Leucine	12.5
Lysine	12.6
Methionine	3.5
Phenylalanine	8.3
Threonine	8.2
Valine	7.4
Dispensable AA	
Alanine	7.4
Aspartic acid	15.2
Cysteine	3.1
Glutamic acid	38.2
Glycine	7.1
Proline	11.4
Serine	8.6
Tyrosine	4.7

*Tryptophan could not be analysed with the described method.
†Calculated according to NRC (1998).

In the present study, supplementation of betaine increased ileal DM digestibility, albeit of small magnitude, which is in agreement with the results of a previous study where addition of betaine-rich condensed molasses solubles to the diet of piglets improved ileal digestibility of DM but had no effect on total tract DM digestibility (Eklund et al., 2006b). In contrast to the results of the present study, supplementation of betaine monohydrate did not improve ileal but faecal DM digestibility in piglets (Eklund et al., 2006b). Higher ileal or faecal DM digestibilities following dietary betaine supplementation have also been reported in previous studies with piglets (Xu and Yu, 2000; Mosenthin et al., 2007). The variable responses to dietary betaine supplementation on DM digestibility in piglets may be attributed to the use of different sources of betaine in the aforementioned studies and because of differences in natural betaine level, especially from wheat. In growing pigs, dietary betaine supplementation had no effect on total tract DM digestibility (Øverland et al., 1999; Fernandez-Figares et al., 2008).

The improvement in fibre digestibility in this study is confirmed by the results of previous studies in piglets, where betaine originating from different sources increased ileal and (or) total tract NDF and ADF digestibilities (Eklund et al., 2006a; b). Another study revealed a tendency for increased ileal or total tract crude fibre digestibilities in piglets following betaine supplementation ranging between 4.7% and 6.5% units (Mosenthin et al., 2007). As pigs produce no fibre degrading enzymes, these results indicate that betaine has the potential to stimulate bacterial fermentation of dietary fibre in the gastrointestinal tract. It is generally accepted that intestinal bacteria may have a requirement for compatible osmolytes such as betaine (Eklund et al., 2005, 2006a; b). Betaine may origin either from dietary sources or bacterial synthesis in the mitochondria (Boch et al., 1994; Simon, 1999; Lewis, 2003). Betaine supplementation may aid the intestinal bacteria to cope with variable osmotic conditions in the gastrointestinal tract.

Concentrations of diaminopimelic acid, ornithine, lactic acid and short-chain fatty acids in ileal digesta and faeces

Ileal concentrations of DAP, which is a constituent of bacterial cell walls (Rowan et al., 1992), were not affected ($p > 0.05$) by dietary betaine supplementation (Table 5). However, supplementation with 1.5 g ($p = 0.11$) or 6.0 g ($p = 0.18$) betaine per kilogram assay diet tended to increase ileal Orn concentrations

Table 3 Effect of graded dietary levels of betaine (g/kg diet, as-fed) on ileal nutrient digestibilities (%)*

	Dietary betaine levels				p-Value†	
Item	0	1.5	3.0	6.0	Linear effect	Quadratic effect
n	2	2	2	2		
Observations	8	8	7	8		
Dry matter	68.9 ± 0.44	70.3 ± 0.44	70.8 ± 0.48	69.5 ± 0.44	0.039	0.027
Crude ash	32.3 ± 2.59	36.2 ± 2.59	36.9 ± 2.74	34.5 ± 2.59	0.630	0.246
Ether extracts	80.8 ± 0.60	81.2 ± 0.60	82.3 ± 0.64	82.0 ± 0.60	0.287	0.354
Crude protein	75.5 ± 1.07	77.4 ± 1.07	77.5 ± 1.13	76.6 ± 1.07	0.567	0.228
NDF	11.3 ± 2.02	18.9 ± 2.02	22.5 ± 2.18	15.2 ± 2.02	0.006	0.002
ADF	0.4 ± 5.36	7.3 ± 5.36	11.2 ± 5.42	2.5 ± 5.36	0.551	0.152

n, number of pigs.
*LS mean values ± SEM.
†p-values for Wald-type *F*-tests for treatment differences.

Table 4 Effect of graded dietary levels of betaine (g/kg diet, as-fed) on total tract nutrient digestibilities (%)*

	Dietary betaine levels					p-Value†	
Item	0	1.5	3.0	6.0	SEM	Linear effect	Quadratic effect
n	2	2	2	2			
Observations	8	8	8	8			
Dry matter	86.8	87.0	87.2	86.7	0.25	0.510	0.140
Crude ash	56.7	57.5	55.1	54.7	1.38	0.510	0.974
Ether extracts	71.7	71.9	71.2	72.7	0.50	0.189	0.267
Crude protein	86.9	87.8	89.2	87.3	0.96	0.328	0.030
NDF	52.9	51.6	55.1	53.2	1.19	0.313	0.564
ADF	33.5	32.2	35.1	32.4	2.21	0.778	0.682

n, number of pigs.
*LS mean values ± SEM.
†p-Values for Wald-type *F*-tests for treatment differences.

compared with the control treatment, whereas there was no effect on ileal Orn concentration when 3.0 g of betaine was supplemented to the diet. Similarly, a previous study showed an increase in Orn concentrations in ileal digesta of piglets fed diets supplemented with betaine (Eklund et al., 2006b). In cell walls of gram positive bacteria, Orn replaces part of DAP (Reverter et al., 1999; Mayer, 2005). Thus, higher ileal Orn concentrations following betaine supplementation may indicate increased activity of the microflora of the small intestine such as Lactobacilli (Mayer, 2005; Eklund et al., 2006b). In support of this observation, D-lactic acid concentrations in ileal digesta increased linearly as dietary betaine level increased ($p < 0.05$). Similarly, betaine addition to the drinking water of broiler chicks increased lactic acid concentrations in the ileum and caecum (Kettunen et al., 1999). However, ileal L-lactic acid, total lactic acid and SCFA concentrations were not affected ($p > 0.05$) in the present study.

The faecal DAP concentrations increased linearly ($p < 0.05$), indicating enhanced bacterial growth as dietary betaine level increased (Table 6). The increase in faecal DAP concentrations was associated with trends towards quadratic increases ($p = 0.06$ to $p = 0.11$) in total tract digestibility of AA (Table 7), indicating increased degradation of AA by bacteria as dietary betaine level increased. In contrast, faecal SCFA concentrations were not affected by dietary betaine supplementation ($p > 0.05$), but they were numerically lower when compared with the control treatment.

Ileal and total tract digestibility of crude ash, ether extract, protein and amino acids

Dietary supplementation of graded levels of betaine did not affect ($p > 0.05$) ileal and total tract digestibilities of CA and EE (Tables 3 and 4). Moreover, there was no effect of betaine supplementation on

Table 5 Effect of graded dietary levels of betaine (g/kg diet, as-fed) on concentrations of bacterial metabolites in ileal digesta (per kg DM)*

	Dietary betaine levels				p-Value†	
Item	0	1.5	3.0	6.0	Linear effect	Quadratic effect
n	2	2	2	2		
Observations	8	8	7	8		
DAP, mg	44.0 ± 4.57	41.3 ± 4.57	42.2 ± 4.92	38.3 ± 4.57	0.841	0.964
Orn, mg	105.9 ± 37.18	192.8 ± 37.18	102.8 ± 40.04	178.6 ± 37.18	0.225	0.930
D-lactic acid, g	1.9 ± 1.25	2.4 ± 1.25	2.6 ± 1.35	6.6 ± 1.25	0.007	0.273
L-lactic acid, g	36.9 ± 9.62	37.5 ± 9.62	37.9 ± 10.01	46.4 ± 9.62	0.878	0.777
Total lactic acid, g	38.2 ± 10.36	39.5 ± 10.36	40.5 ± 10.77	54.1 ± 10.36	0.692	0.673
Acetic acid, mmol	105.8 ± 20.78	98.8 ± 20.78	109.5 ± 21.56	126.9 ± 20.78	0.802	0.671
Propionic acid, mmol	24.9 ± 7.23	19.8 ± 7.23	31.3 ± 7.35	26.5 ± 7.23	0.747	0.834
Isobutyric acid, mmol	0.7 ± 0.23	0.6 ± 0.23	0.6 ± 0.23	0.8 ± 0.23	0.810	0.521
Butyric acid, mmol	6.2 ± 2.96	5.7 ± 2.96	6.8 ± 3.04	12.1 ± 2.96	0.476	0.598
Isovaleric acid, mmol	1.0 ± 0.37	0.7 ± 0.37	0.7 ± 0.38	1.3 ± 0.37	0.653	0.483
Valeric acid, mmol	1.2 ± 0.40	1.0 ± 0.40	1.1 ± 0.41	1.7 ± 0.40	0.636	0.476
Total SCFA, mmol	140.5 ± 33.03	127.1 ± 33.03	152.0 ± 33.38	166.5 ± 33.03	0.853	0.493

n, number of pigs.
*LS mean values ± SEM.
†p-Values for Wald-type *F*-tests for treatment differences.

Table 6 Effect of graded dietary levels of betaine (g/kg diet, as-fed) on concentrations of bacterial metabolites in faeces (per kg DM)*

	Dietary betaine levels					p-Value†	
Item	0	1.5	3.0	6.0	SEM	Linear effect	Quadratic effect
n	2	2	2	2			
Observations	8	8	8	8			
D-lactic acid, g	0.5	0.6	0.3	0.5	0.10	0.277	0.640
L-lactic acid, g	0.6	0.7	0.5	0.7	0.07	0.509	0.293
Total lactic acid, g	1.1	1.3	0.7	1.2	0.13	0.208	0.365
DAP, mg	545.0	575.1	644.0	673.4	46.60	0.040	0.263
Orn, mg	97.6	79.4	89.7	98.6	10.60	0.583	0.243
Acetic acid, mmol	266.8	196.4	219.6	216.0	56.01	0.835	0.416
Propionic acid, mmol	107.0	75.2	72.3	79.9	23.46	0.725	0.353
Isobutyric acid, mmol	13.2	12.7	11.1	13.0	2.17	0.892	0.382
Butyric acid, mmol	44.7	32.0	24.2	37.4	12.15	0.699	0.424
Isovaleric acid, mmol	20.6	20.5	17.4	20.8	3.60	0.893	0.425
Valeric acid, mmol	15.8	12.4	10.6	13.1	0.38	0.762	0.326
Total SCFA, mmol	468.0	349.2	355.2	380.2	99.23	0.821	0.439

n, number of pigs.
*LS mean values ± SEM.
†p-Values for Wald-type *F*-tests for treatment differences.

ileal CP and AA digestibilities except for a linear increase ($p < 0.05$) in ileal digestibility of glycine (Table 3 and 8) as dietary betaine level increased. Glycine is known as a major constituent of bile salt conjugates (Souffrant, 1991). The higher bacterial fermentation of dietary fibre in the small intestine, as indicated by increased ileal NDF digestibility and a trend towards increased ileal Orn concentrations following dietary betaine supplementation may have stimulated bacterial deconjugation of bile salts. Therefore, reabsorption of endogenous glycine may have been facilitated, resulting in improved ileal digestibility of glycine (Jonsson et al., 1995).

In contrast to ileal digestibilities of CP and AA, total tract CP digestibility (Table 4) increased quadratically ($p < 0.05$), and digestibility of AA tended to increase quadratically ($p = 0.06$ to $p = 0.11$), except for proline ($p = 0.28$), as dietary betaine level

Table 7 Effect of graded dietary levels of betaine (g/kg diet, as-fed) on total tract amino acid digestibilities (%)*

Item	Dietary betaine levels				SEM	p-Value†	
	0	1.5	3.0	6.0		Linear effect	Quadratic effect
n	2	2	2	2			
Observations	8	8	8	8			
Indispensable AA							
Arginine	91.3	92.1	93.4	92.2	0.59	0.115	0.067
Histidine	90.2	91.1	92.3	91.1	0.61	0.120	0.063
Isoleucine	85.1	86.2	87.7	86.2	0.86	0.240	0.108
Leucine	86.8	87.8	89.1	88.0	0.71	0.188	0.097
Lysine	89.2	90.1	91.4	90.1	0.67	0.181	0.076
Phenylalanine	87.8	88.6	89.7	88.7	0.63	0.200	0.104
Threonine	86.6	87.7	88.6	87.6	0.66	0.224	0.092
Valine	84.9	86.1	87.7	86.2	0.89	0.197	0.092
Dispensable AA							
Alanine	80.5	82.1	83.7	82.0	0.99	0.203	0.085
Aspartic acid	86.0	87.0	88.4	87.0	0.75	0.177	0.082
Glutamic acid	93.6	94.0	94.8	93.9	0.37	0.184	0.075
Glycine	83.8	85.0	86.0	84.8	0.76	0.260	0.106
Proline	92.6	93.1	93.8	93.3	0.48	0.458	0.281
Serine	88.2	89.2	90.1	89.0	0.56	0.144	0.058
Tyrosine	85.0	86.1	87.5	86.0	0.84	0.215	0.086

n, number of pigs.
*LS mean values ± SEM.
†p-Values for Wald-type *F*-tests for treatment differences.

Table 8 Effect of graded dietary levels of betaine (g/kg diet, as-fed) on ileal amino acid digestibilities (%)*

Item	Dietary betaine levels				p-Value†	
	0	1.5	3.0	6.0	Linear effect	Quadratic effect
n	2	2	2	2		
Observations	8	8	7	8		
Indispensable AA						
Arginine	83.2 ± 0.91	84.9 ± 0.91	84.2 ± 0.93	84.1 ± 0.91	0.645	0.449
Histidine	79.3 ± 0.98	81.2 ± 0.98	80.2 ± 1.04	79.4 ± 0.98	0.566	0.328
Isoleucine	77.9 ± 1.56	80.2 ± 1.56	79.7 ± 1.58	78.7 ± 1.56	0.747	0.367
Leucine	77.8 ± 1.41	80.5 ± 1.41	79.4 ± 1.46	78.7 ± 1.41	0.609	0.362
Lysine	84.6 ± 1.41	85.2 ± 1.41	85.4 ± 1.46	84.3 ± 1.41	0.934	0.586
Phenylalanine	79.6 ± 1.18	81.9 ± 1.18	81.0 ± 1.20	80.6 ± 1.18	0.622	0.398
Threonine	74.4 ± 1.61	76.9 ± 1.61	76.2 ± 1.63	75.2 ± 1.61	0.707	0.328
Valine	74.4 ± 1.72	77.3 ± 1.72	76.3 ± 1.75	74.9 ± 1.72	0.664	0.341
Dispensable AA						
Alanine	72.1 ± 2.13	75.0 ± 2.13	74.4 ± 2.21	72.1 ± 2.13	0.707	0.316
Aspartic acid	74.5 ± 1.30	76.0 ± 1.30	76.0 ± 1.36	74.8 ± 1.30	0.789	0.398
Glutamic acid	86.3 ± 0.82	86.8 ± 0.82	87.4 ± 0.87	86.9 ± 0.82	0.826	0.472
Glycine	61.3 ± 1.19	64.3 ± 1.19	66.5 ± 1.28	66.6 ± 1.19	0.014	0.102
Proline	79.9 ± 1.17	81.7 ± 1.17	81.3 ± 1.21	82.3 ± 1.17	0.558	0.714
Serine	75.6 ± 1.06	77.6 ± 1.06	77.5 ± 1.12	76.1 ± 1.06	0.506	0.163
Tyrosine	78.7 ± 1.49	81.2 ± 1.49	80.3 ± 1.54	79.2 ± 1.49	0.674	0.330

n, number of pigs.
*LS mean values ± SEM.
†p-Values for Wald-type *F*-tests for treatment differences.

increased (Table 7). The highest increase in total tract CP and AA digestibilities was obtained when 3.0 g betaine per kilogram assay diet were supplemented, amounting to 2.3% and 3.2% higher for CP and AA, respectively, compared with the control treatment. These results confirm previous observations according to which betaine improved total tract digestibilities of CP (Eklund et al., 2006a; El-Husseiny et al., 2007; Mosenthin et al., 2007) and AA (Remus et al., 1995; Augustine and Danforth, 1999; Eklund et al., 2006a,b) in piglets and poultry.

In pigs, AA are absorbed only proximal to the distal ileum, whereas digestion and disappearance of AA in the large intestine results from bacterial degradation (Sauer and Ozimek, 1986; Mosenthin and Rademacher, 2003). Therefore, the present data indicate that betaine stimulates the bacterial degradation of CP and AA in the large intestine, which is in accordance with the linear increase in faecal DAP concentrations as dietary betaine level increases. Moreover, the results of this study revealed that betaine supplementation improved ileal but not total tract NDF digestibility. It remains still speculative, however, if due to the lower amount of NDF reaching the large intestine bacteria might have utilised AA as source of energy, thereby stimulating breakdown and disappearance of AA as suggested by Eklund et al. (2006b).

Conclusions

Bacterial fermentation of dietary NDF in the small intestine rather than in the large intestine increased as dietary betaine level increased. However, bacterial degradation of protein and amino acids in the large intestine increased as the dietary betaine level increased. Dietary supplementation of graded levels of betaine enhanced the level of microbial metabolites such as D-lactic acid concentration in ileal digesta and DAP concentration in faeces. In several cases, there was a response to the variables that were measured up to 3.0 g betaine per kilogram diet, however, increasing the dietary betaine level from 3.0 to 6.0 g betaine per kilogram diet had no or only minor effects.

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