Bulletin UASVM, Veterinary Medicine 66(1)/2009
ISSN 1843-5270; Electronic ISSN 1843-5378

Intestinal Effects of Dietary Betaine in Piglets

Rainer MOSENTHIN[1], Adi RATRIYANTO[1,2], Dagmar JEZIERNY[1], Meike EKLUND[1]

[1] Institute of Animal Nutrition, University of Hohenheim, Emil-Wolff-Str. 10, 70599 Stuttgart, Germany; rhmosent@uni-hohenheim.de
[2] Department of Animal Science, University of Sebelas Maret, Surakarta, Indonesia

Abstract. 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 fermentation characteristics in piglets. A total of 8 barrows (BW 7.9 kg) was fitted with simple T-cannulas at the distal ileum. The animals were randomly allocated to 1 of the 4 assay diets with 2 pigs per treatment in 4 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 kg^{-1} diet (as–fed). Ileal digestibilities of dry matter (DM) and neutral detergent fiber (NDF) increased both quadratically and linearly ($P<0.05$), and ileal digestibility of glycine increased linearly as dietary betaine level increased ($P<0.05$). Moreover, there were linear increases in the concentrations of ileal D–lactic acid ($P<0.05$), indicating intensified intestinal bacterial activities as dietary betaine level increased. At the fecal level, total tract crude protein (CP) digestibility increased quadratically ($P<0.05$), and digestibility of amino acids (AA) tended to increase quadratically ($P=0.06$ to $P=0.11$), except for proline ($P>0.05$), as dietary betaine level increased. The increased bacterial degradation of CP and AA in the large intestine coincides with the linear increase ($P<0.05$) in fecal diaminopimelic acid concentrations, indicating enhanced intestinal bacterial growth with increasing dietary betaine levels. In most cases, there was a response in the variables that were measured up to 3.0 g betaine per kg diet, whereas increasing the betaine level from 3.0 to 6.0 g betaine per kg diet had no additional effect. It can be concluded that dietary betaine stimulates microbial fermentation of fiber in the small intestine, leaving less fermentable fiber to reach the large intestine and therefore, increased microbial degradation of protein in the large intestine may occur.

Keywords: piglets, betaine, digestibility, bacterial fermentation, microflora

INTRODUCTION

Over the last years, considerable research has been carried out to study the effect of supplemental betaine on growth performance, carcass composition and intestinal health of pigs. Supplementation of betaine to the diet improved weight gain and feed conversion in pigs (Dunshea *et al.*, 2007; Wray-Cahen *et al.*, 2004). Improvements in growth performance of pigs due to dietary betaine supplementation may, at least in part, be attributed to improved nutrient digestibilities (Eklund *et al.*, 2006a, b; Xu and Yu, 2000).

Due to its osmoprotective properties, betaine supplementation to piglet diets has been shown to improve digestibility of fiber (Eklund *et al.,* 2006a, b; Ratriyanto *et al.,* 2007), whereas the effects on CP digestibility were equivocal (Eklund *et al.,* 2006b). Higher fiber digestibility following betaine supplementation has been attributed to increased bacterial fermentation of dietary fiber. It has not yet been clarified, if higher ileal CP and AA digestibilities following betaine supplementation can be attributed either to improved enzymatic digestion and a higher absorption capacity of the intestinal epithelium or to intensified microbial degradation of protein (Eklund *et al.,* 2006b). Furthermore, decreased digestibility of CP indicated intensified bacterial assimilation of CP due to betaine supplementation (Eklund *et al.,* 2006b). Moreover, between studies, there exist considerable variations in the level of betaine supplementation to pig diets, ranging between 0.02 and 0.50% (e.g. Fernandez-Figares *et al.,* 2002; Hur *et al.,* 2007), and betaine effects between studies were of different magnitude. Thus, the present study aimed to determine the effects of graded levels of dietary betaine supplementation on ileal and total tract nutrient digestibilities and intestinal microbial fermentation characteristics in piglets.

MATERIALS AND METHODS

The experiment was carried out with 8 five-week-old barrows (German Landrace × Piétrain). The piglets were surgically fitted with a simple T-cannula at the distal ileum on d 7 and 9 after arrival according to the principles described by Li *et al.* (1993), and adhesive collection bags were attached to the pigs' anus for collection of feces. The pigs were individually housed in stainless-steel metabolic crates. The basal diet consisted of wheat, barley, soybean meal, a mineral and vitamin premix and titanium dioxide (TiO_2) as an indigestible marker (Table 1). The diet was formulated to meet the nutrient requirements of NRC (1998) for piglets from 10 to 20 kg of BW. The liquid betaine product (25% betaine content) was added to the basal diet at the expense of cornstarch. Four experimental diets with graded dietary inclusion levels of betaine were formulated:

CON	: basal diet ('Control')
BET 0.15	: basal diet plus 0.15% betaine (0.60% liquid betaine product)
BET 0.30	: basal diet plus 0.30% betaine (1.20% liquid betaine product)
BET 0.60	: basal diet plus 0.60% betaine (2.40% liquid betaine product)

The animals were fed 2 equal meals daily at 07.00 and 19.00 h at a level of 4.5% of their individual BW. The meals were offered in mash form, and were mixed with water (1/1 w/v). The pigs had free access to water. Following a 7–d adaptation to the experimental diets after surgery, the collection of feces was initiated at 07.00 h on d 8 and continued for 72 consecutive hours. Ileal digesta were collected for 2 x 12 h, from 07.00 to 19.00 h on d 11 and from 19.00 to 07.00 h on d 12 of each period. Between each feces and digesta collection there were 5 days adaptations to the new feed allowances. 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 sampling tubing in order to minimise further bacterial fermentation in digesta, except for the samples for the determination of volatile fatty acid (VFA) and lactate concentrations. Ileal and fecal samples were pooled and mixed within animal and period, and were freeze-dried thereafter. Samples of diets, ileal digesta and feces were milled through a 1.0 mm mesh screen prior to analyses.

Determination of DM, crude ash (CA), CP, ether extracts (EE), NDF, acid detergent fiber (ADF) and AA was performed as outlined by Naumann and Bassler (1997). The D– and

L– lactate concentrations were determined by means of a photometric test kit (Boehringer, No. 1 112 821). The VFA concentrations were measured by gas chromatography using 4–methyl–iso–valerianic acid as internal standard. Samples for VFA analyses were prepared according to the principles described by Zijlstra *et al.* (1977). The TiO_2 contents in feed, ileal digesta and fecal samples were determined according to the procedures described by Brandt and Allam (1987).

Initially, the following linear model for selecting a repeated correlation structure was considered: $y_{ijk} = \mu + \beta_j + \delta_i + \beta_j \times \delta_i + e_{ijk}$ where y_{ijk} = j^{th} measurement on k^{th} animal in i^{th} betain level, μ = general term (fixed), β_j = effect of j^{th} period (fixed), δ_i = effect of i^{th} 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 modeled 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

Ileal digestibilities of DM and NDF increased, both quadratically ($P<0.05$) and linearly ($P<0.05$) as dietary betaine level increased (Tab. 1). The highest increase in ileal DM and NDF digestibilities was obtained when 3.0 g betaine per kg assay diet was supplemented, amounting to 1.9 and 11.2 percentage units 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 kg assay diet had no additional effect on ileal digestibilities of DM or NDF.

Tab. 1

Effect of graded dietary levels of betaine (g kg^{-1} diet, as–fed) on ileal nutrient digestibilities (%)[*]

					P–values[†]	
	Dietary betaine levels				Linear effect	Quadratic effect
Item	0	1.5	3.0	6.0		
n (pigs)	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

[*] LS mean values ± SEM.

[†] P–values for Wald–type F–tests for treatment differences.

The improvement in fiber digestibility in this study is confirmed by the results of previous studies in piglets, according to which betaine originating from different sources increased considerably ileal and (or) total tract NDF and ADF digestibilities from 8.7 to 17.9 percentage units (Eklund *et al.,* 2006a, b). Another study revealed a tendency for increased ileal or total tract crude fiber digestibilities in piglets following betaine supplementation ranging between 4.7 and 6.5 percentage units (Mosenthin *et al.*, 2007). As pigs in general lack fiber degrading enzymes, these results indicate that betaine has the potential to stimulate bacterial fermentation of dietary fiber in the gastrointestinal tract. It has been suggested that intestinal bacteria may have a requirement for compatible osmolytes such as betaine (Eklund *et al.*, 2005, 2006a, b). Betaine supplementation may aid the intestinal bacteria to cope with the various osmotic conditions in the gastrointestinal tract, allowing for enhanced intestinal bacterial fermentation of dietary fiber as dietary supplementation of graded levels of betaine did not affect (P>0.05) ileal and total tract digestibilities of CA and EE. Moreover, there was no effect of betaine supplementation on ileal CP and AA digestibilities except for a linear increase (P<0.05) in ileal digestibility of glycine as dietary betaine level increased (data not shown).

Tab. 2

Effect of graded dietary levels of betaine (g kg^{-1} diet, as–fed) on total tract amino acid digestibilities (%)[*]

						P–values[†]	
	Dietary betaine levels					Linear	Quadratic
Item	0	1.5	3.0	6.0	SEM	effect	effect
n (pigs)	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

[*] LS mean values.

[†] P–values for Wald–type F–tests for treatment differences.

In contrast to ileal digestibilities of CP and AA, total tract CP digestibility 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 increased (Tab. 2). The highest increase in total tract CP and AA digestibilities was obtained when 3.0 g betaine per kg assay diet were supplemented, amounting to 2.3 and 3.2 percentage units for CP and AA, respectively, compared with the control treatment. These results confirm previous observations in piglets according to which betaine improved total tract digestibilities of CP and AA (Eklund *et al.,* 2006a, b; Mosenthin *et al.,* 2007). In pigs, AA are absorbed proximal to the distal ileum only, whereas digestion and disappearance of AA in the large intestine results from bacterial degradation (Mosenthin and Rademacher, 2003; Sauer and Ozimek, 1986). Therefore, the present data indicate, that betaine stimulates the bacterial degradation of CP and AA in the large intestine. Accordingly, there were linear increases in the concentrations of ileal D–lactic acid and of fecal diaminopimelic acid ($P<0.05$), indicating intensified intestinal bacterial activities as dietary betaine level increased. 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

In conclusion, bacterial fermentation of dietary fiber in the small intestine rather than in the large intestine increased as dietary betaine level increased. On the other hand, bacterial degradation of CP and AA in the large intestine increased as the dietary betaine level increased. Changes in bacterial metabolite concentrations and bacterial marker levels, both in ileal digesta and in feces, indicate a shift in the composition of the bacterial community due to dietary betaine supplementation.

REFERENCES

1. Brandt, M. and S. M. Allam (1987). Analytik von TiO2 im Darminhalt und Kot nach Kjeldahlaufschluß. Arch. Anim. Nutr. 37:453–454.
2. Dunshea, F. R., D. J. Cadogan and G. G. Partridge (2007). Dietary betaine and rectopamine have additive effects on lean tissue deposition, particularly in restrictively-fed gilts. In: Manipulating Pig Production XI. (Ed. J.E. Paterson and J.A. Barker). Australasian Pig Science Association, Scott Print, Perth, Western Australia. pp. 120.
3. Eklund, M., E. Bauer, J. Wamatu and R. Mosenthin (2005). Potential nutritional and physiological functions of betaine in livestock. Nutr. Res. Rev. 18:31–48.
4. Eklund, M., R. Mosenthin, M. Tafaj and J. Wamatu (2006a). Effects of betaine and condensed molasses solubles on nitrogen balance and nutrient digestibility in piglets fed diets deficient in methionine and low in compatible osmolytes. Arch. Anim. Nutr. 60:289–300.
5. Eklund, M., R. Mosenthin and H.-P. Piepho (2006b). Effects of betaine and condensed molasses solubles on ileal and total tract nutrient digestibilities in piglets. Acta Agric. Scand. Section A 56:83–90.
6. Fernandez-Figares, I., D. Wray-Cahen, N. C. Steele, R. G. Campbell, D. D. Hall, E. Virtanen and T. J. Caperna (2002). Effect of dietary betaine on nutrient utilization and partitioning in the young growing feed-restricted pig. J. Anim. Sci. 80:421–428.
7. Hur, S. J., H. S. Yang, G. B. Park and S. T. Joo (2007). Effects of dietary glycine betaine on pork quality in different muscle types. Asian-Aust. J. Anim. Sci. 20:1754–1760.
8. Li, S., W. C. Sauer and M. Z. Fan (1993). The effect of dietary crude protein level on amino acid digestibility in early–weaned pigs. J. Anim. Physiol. a. Anim. Nutr. 70:26–37.
9. Mosenthin, R. and M. Rademacher (2003). Digestible amino acids in diet formulation for pigs. In: D'Mello, J. P. F. (ed.), Amino Acids in Animal Nutrition. CABI Publishing, Wallingford, Oxon, UK, pp. 169–196.
10. Mosenthin, R., E. Eklund and A. Ratriyanto (2007). Effects of betaine on ileal and faecal digestibilities and intestinal microbial fermentation of crude fibre in piglets. In: Paterson, J. E., J. A. Barker (eds.), Manipulating Pig Production XI. Scott Print, Perth, Western Australia. p. 187.
11. Naumann, C. and R. Bassler (1997). Die chemische Untersuchung von Futtermitteln. VDLUFA–Verlag, Darmstadt, Germany.
12. NRC, National Research Council (1998). Nutrient Requirement of Swine. 10th edn. National Academic Press, Washington, DC, USA.
13. Ratriyanto, A., M. Eklund and R. Mosenthin. (2007). Effects of betaine supplementation on ileal and fecal digestibilities and intestinal microbial fermentation of nutrients in piglets. Proceedings International Scientific Symposium on Physiology of Livestock. Kaunas. Lithuania. pp. 34–35.
14. SAS (2003). SAS User's Guide: Statistics, Version 9. SAS Inst. Inc., Cary, NC, USA.
15. Sauer, W. C. and L. Ozimek (1986). Digestibility of amino acids in swine: results and their practical applications. A review. Livest. Prod. Sci. 15:367–388.

16. Wray-Cahen, D., I. Fernández-Fígares, E. Virtanen, N. C. Steele and T. J. Caperna (2004). Betaine improves growth, but does not induce whole body or hepatic palmitate oxidation in swine (Sus scrofa domestica). Comp. Biochem. Physiol. Part A. 137:131–140.

17. Xu, Z. R. and D. Y. Yu (2000). Effect of betaine on digestive function of weaned piglets. Chin. J. Vet. Sci. 20:201–204.

18. Zijlstra, J. B., J. Beukema, B. G. Wolthers, B. M. Byrne, A. Groen and J. Dankert (1977). Pretreatment methods prior to gaschromatographic analysis of volatile fatty acids from faecal samples. Clinica Chimica Acta 78:243–250.