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Betaine increases net portal absorption of volatile fatty acids in Iberian pigs

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ABSTRACT

Betaine is an osmolyte with the potential to increase volatile fatty acids (**VFAs**) production and hence improve intestinal health.The present study investigated how betaine affects portal and arterial concentrations and net portal absorption (**NPA**) of VFA in growing Iberian pigs. Eight 30 kg BW Iberian growing barrows with indwelling catheters in portal vein, ileal vein and carotid artery were randomly assigned to a control diet or a diet supplemented with 0.5% betaine. Para-aminohippuric acid was infused into the ileal vein as a marker to determine portal blood flow using the dilution method. Blood samples were simultaneously taken from the carotid artery and portal vein at −60, 60, 120, 180, 240, 300 and 360 min after feeding 1 200 g of the diet. The NPA of VFA (acetate, propionate, butyrate, valerate, isobutyrate and caproate) was determined by multiplying the porto-arterial plasma concentration differences by portal plasma flow. Betaine increased NPA of acetate (1.44 fold; $P < 0.001$) and total VFA (0.55 fold; $P < 0.001$) while decreased NPA of propionate (−0.38 fold; $P < 0.05$) and valerate (−1.46 fold; $P < 0.05$) compared with control pigs. Estimated heat production potentially derived from NPA of VFA accounted for 0.20–0.27 of metabolizable energy for maintenance. Acetate and propionate accounted for most of the total VFA estimated heat production (0.83–0.89). Regarding bacterial communities, betaine apparently did not change the DNA abundance of fecal total bacteria, *Lactobacillus*, *Bifidobacterium*, Enterobacteriaceae, *Bacteroides* and the *Clostridium* clusters I, IV and XIV. In conclusion, betaine increased portal appearance and NPA of VFA, contributing to cover maintenance energy requirements.

Implications

Betaine is a natural additive used as growth promoter in swine. We have showed that betaine boosts volatile fatty acid net portal absorption in young pigs, which may be beneficial for gut health and as an extended source of energy for the animal.

Introduction

A typical cereal based pig diet contains significant quantities of fermentable carbohydrates which are substrates for bacterial fermentation mainly in the large intestine. The products of fiber fermentation in the large intestine are volatile fatty acids (**VFAs**), which positively affect gut health (Lindberg, 2014). Moreover, the VFAs are energy fuels of ceco-colonic epithelial cells, liver and muscle cells (Roberfroid, 2007). In fact, it has been estimated that VFA may account for up to 24% of pig heat production (Yen et al., 1991) if all the absorbed VFAs into the portal vein are combusted to CO_2.

Betaine (trimethyl glycine) is a quaternary amine with three chemically reactive methyl groups linked to the nitrogen atom and it is widespread in animals and plants (marine invertebrates, sugar beet, wheat middlings and spinach). It is a methyl donor and a compatible osmolyte that could favor nutrient digestibility helping microbiota and enterocytes to deal with varying osmotic conditions along the digestive tract (Eklund et al., 2006). Some studies have proved that betaine increases DM and fiber digestibility in piglets (Eklund et al., 2006; Ratriyanto et al., 2009) and modifies intestinal microbiota (Eklund et al., 2006) increasing bacterial activity (Ratriyanto et al., 2009). Interestingly, certain bacterial taxa in the colon of mice metabolized betaine into other betainized compounds (Koistinen et al., 2019) may be mediating the metabolic effect of diet and colonic microbiota. Moreover, we have evi-

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dences that betaine affects *in vitro* fermentation capacity of pigs (Pardo et al., 2020). After the ban of in-feed antibiotic growth promoters in the European Union, there has been a greater use of substances with the potential to increase VFA production and hence improve intestinal health. Although betaine has been used as growth promoter having an impact on body composition in pigs (Fernández-Fígares et al., 2002), positive effects on microbiota and net portal absorption of VFA remain unexplored to date.

We hypothesized that supplementation of betaine to a standard cereal diet would increase VFA net portal absorption in growing pigs. The aim of the present work was to measure net portal absorption (**NPA**) of VFA in multicatheterized pigs fed a betaine diet.

Material and methods

Animals and experimental design

The experiment was undertaken according to Directional Guides Related to Animal Housing and Care (Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes) and all procedures were approved by the Bioethical Committee of the Council for Scientific Research of Spain (CSIC, Madrid, Spain). Eight Iberian (Silvela strain) barrows (*Sus scrofa mediterraneus*; 19 ± 0.3 kg BW) were allocated in individual pens within an environment room where temperature was controlled (21 ± 1.5 °C). The pig supplier was Sánchez Romero Carvajal (Jabugo SA, Sevilla, Spain). Pigs were fed a single meal (0900 h) at 2.8 × metabolizable energy (**ME**) for maintenance. Two experimental diets were formulated. Control diet was barley- and soybean meal-based containing an optimal amino acid profile, 151 g CP/kg DM and 14.5 MJ ME/kg DM). The betaine diet was formulated supplementing the control diet with 5 g/kg (w/w) betaine (Betafin S1, crystalline, 96% purity; Danisco, Copenhagen, Denmark) at the expense of maize starch. Composition and chemical analysis of the diets (Table 1) were performed following Association of Official Analytical Chemists (AOAC, 2000) protocols for DM (no. 934.01) and ash (no. 942.05). Nitrogen content was determined using a LECO Truspec CN determinator (LECO Corporation, St Joseph, MI, USA) following the Dumas procedure, and CP was calculated as total N multiplied by the 6.25 factor. An isoperibolic bomb calorimeter (model PARR 1356, Moline, IL, USA) was used to determine gross energy content of diets. The NDF content in the diets was determined following the procedure of Goering and Van Soest (1970) using a FibertecTMM6 system (Foss Analytical, Hillerød, Denmark). For the NDF analysis, heat-stable amylase was added and all results were expressed as ash-free.

Surgeries to implant catheters were carried out when pigs attained about 34 kg BW (35.2 and 32.1 kg for control and betaine, respectively). Pigs were fasted 24 h before surgery and fitted with chronic indwelling catheters in the portal and ileal veins, and the carotid artery as previously described (Rodríguez-López et al., 2013). Pigs were offered 0.25, 0.60 and 1.00 of their presurgery daily feed ration, respectively, on days 1, 2 and 3 after catheterization. After surgery recuperation (10 days on average), a pulse dose (15 ml) of para-aminohippuric acid (**PAH**; 20 g/kg wt/vol; Sigma-Aldrich Química S.A., Madrid, Spain), which is used as a marker to measure blood flow, was injected into the ileal vein 45 min before blood sampling and a continuous infusion followed up till the end of the sampling (Yen and Killefer, 1987). The PAH was infused using a syringe pump (Model 33, Harvard Apparatus Inc., Holliston, MA, USA) fitted with apyrogenic filters (MILLEX GP, Syringe Driven Filters Unit, 0.22 μm; Millipore, Carringtwohill, Ireland). Blood samples (4.5 ml) were simultaneously taken from the portal vein and the carotid artery at −60, 60, 120, 180, 240, 300 and 360 min after feeding 1 200 g of diet. A microcentrifuge was used (11 500*g* for 5 min; Biocen, Orto-Alresa, Ajalvir, Madrid, Spain) to determine hematocrit. Plasma was obtained by centrifugation (4 °C, 1 820*g* for 30 min; Eppendorf 5810 R, Hamburg, Germany) and stored in aliquots at −20 °C for analysis of PAH (Fernández-Fígares et al., 2018) and VFA.

Table 1
Composition and chemical analysis (as fed) of experimental diets fed to growing Iberian pigs.

	Control	Betaine
Composition (g/kg)		
Barley	840	840
Soybean meal	80	80
Maize starch	32.8	27.8
Betafin S1	-	5
Sunflower oil	10	10
$CaCO_3$	11	11
$CaHPO_4$	12	12
NaCl	5	5
L-Lysine	4	4
DL-Threonine	1	1
BHT	0.125	0.125
Minerals and vitamins[1]	3	3
Chemical analysis (g/kg)		
DM	901	905
Ash	49.5	51.6
CP	139	133
NDF	158	153
ADF	44.5	40.7
Lignin	6.4	5.6
Hemicellulose	113.5	112.3
Gross energy (MJ/kg)	16.3	16.6
Metabolizable energy (MJ/kg)	13.0	13.2

BHT = Butylated hydroxytoluene.
[1] Supplied (per kg diet) 75 mg of iron as $FeSO_4$, $7H_2O$; 15 mg of manganese as $MnSO_4$, $4H_2O$; 450 μg of iodine as KI; 120 mg of zinc as ZnO; 60 mg of copper as $CuSO_4$, $5H_2O$; 300 μg of cobalt as $CoSO_4$, $7H_2O$; 2 253 IU of cholecalciferol; 9 836 IU of vitamin A as retinyl acetate; 1.5 mg of menadione sodium bisulfite; 2.52 IU of vitamin E as DL-α-tocopheryl acetate; 3 mg of riboflavin; 0.15 mg of thiamine; 15 μg of cyanocobalamin; 0.15 mg of pyridoxine; 15 μg of folic acid; 15 mg of D-pantothenic acid as calcium pantothenate; 22.5 mg of nicotinic acid.

Flux of volatile fatty acids

Plasma concentration of acetate, propionate, butyrate, valerate, isobutyrate and caproate was determined after deproteinization (anhydrous ethanol) and centrifugation. The supernatant was made alkaline and dried in a vacuum centrifuge. Immediately before being injected into the gas chromatograph, samples were dissolved into 62.5 g/kg (w/w) orthophosphoric acid and injected into a Shimadzu GC 2010 equipped with a flame ionization detector (FID) and using a 30 m × 0.53 mm × 1 μm semi-capillar column (Supelco, Barcelona, Spain). Injector, detector and oven temperatures were 240, 240 and 140 °C, respectively. A multilevel calibration (0, 2.5, 5, 7.5, 10, 12.5 and 15 mM) was used for VFA quantification and crotonic acid was used as an internal standard.

Portal plasma flow (**PPF**) was determined by the indicator dilution method using portal vein hematocrit and plasma PAH concentrations as described previously by Rodríguez-López et al. (2013). The NPA of VFA was calculated according to the Fick principle by multiplying the porto-arterial plasma concentration difference of the VFA by PPF.

Potential contribution of VFA to energy requirements was calculated multiplying net portal flux of each VFA extrapolated to 24 h by the energy value of each VFA:

$$([\mathrm{VFA}]_{\mathrm{Portal}}, \mathrm{mol/l} - [\mathrm{VFA}]_{\mathrm{Arterial}}, \mathrm{mol/l}) \times \mathrm{PPF}, \mathrm{l/d}, \times \mathrm{Energy\ value\ of\ VFA}, \mathrm{kJ/mol}$$

Daily net portal flux of each VFA was extrapolated from the VFA flux measured along the 6 h sampling period. Energy values for each VFA were (CRC, 2004) 874, 1 527, 2 180, 2 837, 2 180 and 3 351 kJ/mol, respectively, for acetic, propionic, butyric, valeric, isobutyric, and caproic acids.

Microbial analysis

Fecal samples were obtained directly from the anus of each pig the day after blood sampling, homogenized and stored at −80 °C until analysis. The DNA was extracted from thawed feces (220 mg) using the QIAamp DNA Stool Mini Kit (QIAgen, Valencia, CA) following the procedure of Yu and Morrison (2004) except that samples were treated with cetyltrimethylammonium bromide to remove PCR inhibitors. A Nanodrop ND-1000 (Nano-Drop Technologies, Wilmington, DE) was used to quantify eluted DNA. Extracted DNA was used to quantify total bacteria, *Lactobacillus*, *Bifidobacterium*, Enterobacteriaceae, *Bacteroides* and the *Clostridium* clusters I, IV and XIV by quantitative PCR. Thermocycling was conducted in a QuantStudio 1 Thermal Cycler (Applied Biosystem, Foster City, CA, USA) and started with an initial cycle of denaturation (95 °C for 10 min), followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. To determine the specificity of amplification, analysis of product melting was conducted after each amplification. Each PCR reaction mixture (20 μL final volume) contained 8 μL SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK), 0.3 μL of 10 μM each primer, 0.12 μL of Rox (1:10, Thermo Scientific), 9.28 μL of DNase-free water, and 2 μL of extracted DNA. Primer sequences for each bacterial group are detailed elsewhere (Miranda Hevia, 2018 and supplementary material S1).

For quantitative PCR (**qPCR**) quantification of the different bacterial groups, bacterial type strains were used to prepare calibration standards using cultures of the different species at specified concentrations (Supplementary material S1). DNA of these cultures was extracted as described for fecal samples. For each bacterial group, serial dilutions of the DNA obtained were run in each qPCR plate together with the fecal samples.

Statistical analysis

The experimental unit was the pig. Sequential measurements were made over time in each pig. A multivariate ANOVA with PROC MIXED of SAS for repeated measures was carried out. The main effects in the model were the diet, sampling time and their interaction.

$$X_{ijk} = \mu + \alpha_i + \beta_j + v_k + (\alpha v)_{ik} + \varepsilon_{ijk}$$

where α_i is the diet, β_j is the pig, v_k is time, and $(\alpha v)_{ik}$ is the two-factorial interaction between diet and time. The error term ε_{ijk} represents unexplained variation. Bacterial concentration and percentages were analyzed using the same model excluding the effect of time. The differences were considered significant when $P < 0.05$ and a trend when $P < 0.10$.

Sample size was *a priori* determined with G*Power software (Heinrich-Heine-Universität Düsseldorf) using the variability associated to portal blood flow reported in previous experiments (SD observed was 62 ml/min; Lachica et al., 2019), a type I error probability $\alpha = 0.05$ and a type II error probability $\beta = 0.20$. The minimum portal blood flow difference we wanted to detect was 130 ml/min.

Results

Pigs were restrictively fed (approximately 70% *ad libitum*; Nieto et al., 2012) so there were no feed intake differences among treatments ($P > 0.10$). After surgery, pigs showed good condition and appetite. Growth performance of pigs did not differ between diets (385 g/d on average; $P > 0.10$). Table 2 shows mean portal and arterial concentration (μM) of individual and total VFA. Fig. 1 shows the evolution of portal and arterial concentration of acetate and total VFA along the sampling period. Betaine increased portal and arterial acetate concentration (2.67 and 4.67 fold, respectively; $P < 0.001$) as well as the sum of all VFA (1.86 and 4.21 fold, respectively; $P < 0.001$). Additionally, betaine increased portal caproate ($P < 0.01$) and arterial valerate ($P < 0.01$). Postprandial portal and arterial concentrations of VFA were not different from preprandial concentrations ($P > 0.10$). Portal concentration of individual and total VFA were greater compared with arterial blood. Acetate accounted for 0.70–0.90 of total VFA in portal blood for control and betaine diet, respectively; while in arterial blood, acetate explained 0.89–0.97 of total VFA for control and betaine diet, respectively. Propionate accounted for 0.22 and 0.07 of total VFA in portal blood for control and betaine diet, respectively. In arterial blood, propionate represented only 0.04 and 0.02 of total VFA for control and betaine diet, respectively.

Isovalerate was not quantified because of coelution with an unknown peak.

Table 3 shows the PPF and average NPA of VFA. The PPF was lower (0.79 of control; $P < 0.05$) in pigs fed betaine compared with control. Additionally, PPF increased after the meal ($P < 0.05$), peaked at 60 min (840 ml/min on average) and returned to base line at 300 min (666 ml/min on average) remaining stable until the end of the sampling period. No significant ($P > 0.10$) interactions existed between diets and time of sampling. Betaine increased NPA of acetate and total VFA (1.44 and 0.54 fold, respectively; $0.01 < P < 0.05$) while decreased NPA of propionate, isobutyrate and valerate (-0.38, −0.58 and −1.46 fold, respectively; $0.001 < P < 0.05$) compared with control diet. Acetate and propionate together represented 0.91–0.96 of NPA of total VFA. The NPA of individual VFA was essentially constant through time; the evolution of acetate and NPA of total VFA along the sampling period is shown in Fig. 2.

Heat production values derived from NPA of VFA (Table 3) accounted for 0.20–0.27 of ME for maintenance. Total VFA heat production was greater for betaine (0.29 fold; $P < 0.05$) compared with control diet. Acetate and propionate accounted for most of total VFA heat production (0.83–0.89 of total VFA heat production) for all treatments.

Table 2
Mean portal and arterial concentration of volatile fatty acids (VFAs) in Iberian pigs ($n = 4$/diet) after feeding a diet supplemented or not with betaine (5 g/kg w/w).

				P-value		
	Control	Betaine	SEM	Diet	Time	Diet × Time
Portal (μmol/L)						
Acetate	966	3 546	314	<0.001	0.995	0.987
Propionate	302	283	22	0.556	0.363	0.977
Butyrate	71	80	8.3	0.452	0.951	0.974
Valerate	26	21	2.2	0.103	0.141	0.985
Isobutyrate	12	16	1.4	0.061	0.075	0.999
Caproate	5.0	9.3	0.99	0.004	0.238	0.812
Total VFA	1 382	3 955	313	<0.001	0.996	0.990
Arterial (μmol/L)						
Acetate	530	3 004	225	<0.001	0.481	0.872
Propionate	26	50	14	0.247	0.527	0.523
Butyrate	15	18	1.7	0.223	0.260	0.409
Valerate	11	25	3.4	0.005	0.024	0.725
Isobutyrate	12	12	1.3	0.724	0.558	0.334
Caproate	2.2	2.4	0.34	0.622	0.206	0.961
Total VFA	597	3 111	198	<0.001	0.820	0.879

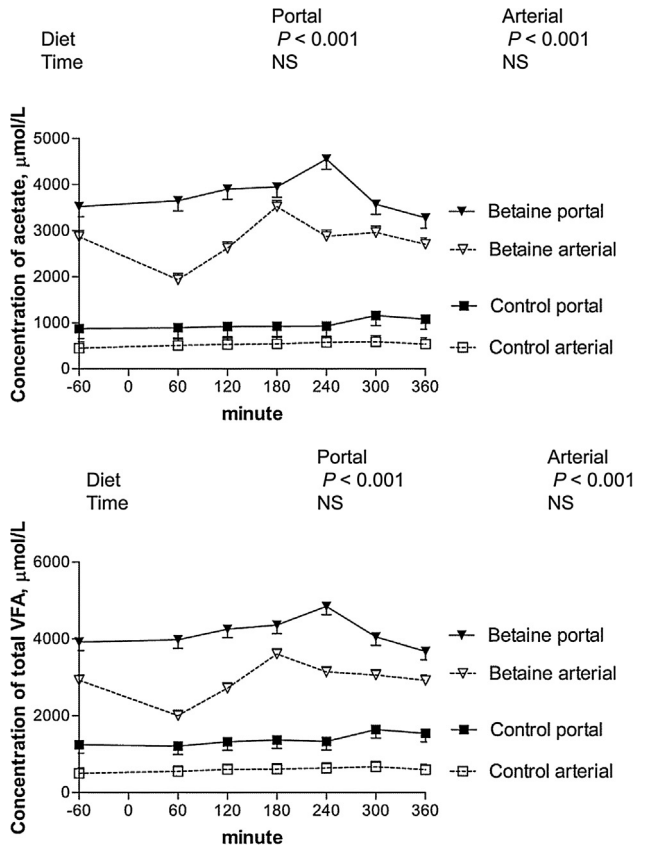

Fig. 1. Portal and arterial concentrations of acetate and total volatile fatty acids (VFAs) in Iberian pigs fed control (■) or betaine (▲) diets. Values are means of four pigs. SEM: acetate = 208; total VFA = 221. NS: Not significant, $P > 0.10$.

Table 4 shows the results of the bacterial community assessment. No differences were observed ($P > 0.10$) in the abundance of total bacteria or the different bacterial groups assessed (*Lactobacillus*, *Bifidobacterium*, Enterobacteriaceae, *Bacteroides* and the *Clostridium* clusters I, IV and XIV). In the same way, no differences ($P > 0.10$) were detected when the proportions of these bacterial groups were calculated related to total bacteria.

Discussion

The VFAs are synthesized mainly in the large intestine (Montoya et al., 2016) as a result of fermentation of substrates that escaped enzymatic hydrolysis in the upper gut. Although digestibility is a measurement of nutrient disappearance in the gut, it does not allow for assessing the postprandial time course of nutrient release, which is of particular importance to evaluate nutrient absorption by the pig (Rerat, 1980). Estimating nutrient appearance in the portal and systemic blood may provide an accurate measurement of nutrient absorption rather than disappearance from the gut lumen (Yen and Killefer, 1987). Interestingly, 90–98% of the produced VFAs are rapidly absorbed by colonocytes in pigs (Montoya et al., 2016). The NPA of VFA reported in this study does not represent total transfer or flux of VFA from the gut lumen because a portion of the absorbed VFA is metabolized by the gastrointestinal mucosa (Yen et al., 1991). According to Rérat et al. (1987), the synthesis and absorption of VFA in the digestive tract of pigs is a continuous process and it is not possible to establish a zero time. The lack of a postprandial peak in portal concentration and NPA of VFA is in line with a continuous fermentation process in the large intestine and may be interpreted as a uniform net portal uptake of VFA. Indeed, we found no differences between preprandial and postprandial concentrations of VFA in accordance with previous studies (Yen et al., 1991; Michel and Rérat, 1998).

The period of sampling in the present experiment was 6 h so we assume that a proportion of the indigestible fraction of the previous day meal was present in the large intestine and mixed with the one offered on the day of sampling (Rérat et al., 1987). Identical sampling time was used to study net portal absorption of VFA in 21 kg crossbred and Meishan barrows fed high fiber diets (Yen et al., 2004).

Portal and arterial VFA concentrations of pigs fed the control diet were within the range of values found in growing-finishing pigs (van der Meulen et al., 1997; Agyekum et al., 2016). Betaine increased portal concentration of total VFA indicating increased fermentability of the diet. The production of VFA varies with the type and amount of fiber and carbohydrates in the diet (Michel and Rérat, 1998) and increases with fiber concentration in the diet (Serena et al., 2009). In our study, both groups of pigs had access to essentially the same barley-corn-soybean meal diet, so the differences reported could not be due to divergent dietary composition.

Table 3
Mean portal plasma flow (PPF), net portal appearance (NPA) of volatile fatty acids (VFAs) and potential heat production associated to VFA oxidation in Iberian pigs ($n = 4$/diet) after feeding a diet supplemented or not with betaine (5 g/kg w/w).

				P-value		
	Control	Betaine	SEM	Diet	Time	Diet × Time
PPF (ml/min)	848	666	25	0.018	0.171	0.522
NPA (µmol/min)						
Acetate	332	810	79	0.003	0.686	0.786
Propionate	266	165	23	0.033	0.575	0.895
Butyrate	39	44	4.4	0.572	0.986	0.989
Valerate	13	−6	1.8	<0.001	0.008	0.056
Isobutyrate	6.7	2.8	0.73	0.010	0.891	0.949
Caproate	2.0	2.4	0.50	0.688	0.635	0.046
Total VFA	659	1 018	85	0.040	0.807	0.820
Heat production of VFA (kJ/day)						
Acetate	417	1 020	70	<0.001	0.712	0.782
Propionate	584	364	40	0.034	0.024	0.859
Butyrate	124	140	13	0.437	0.773	0.999
Valerate	54	19	4.1	<0.001	0.312	0.591
Isobutyrate	17	9	1.3	0.002	0.859	0.952
Caproate	16	19	1.6	0.001	0.906	0.274
Total VFA	1 212	1 562	137	0.013	0.574	0.874

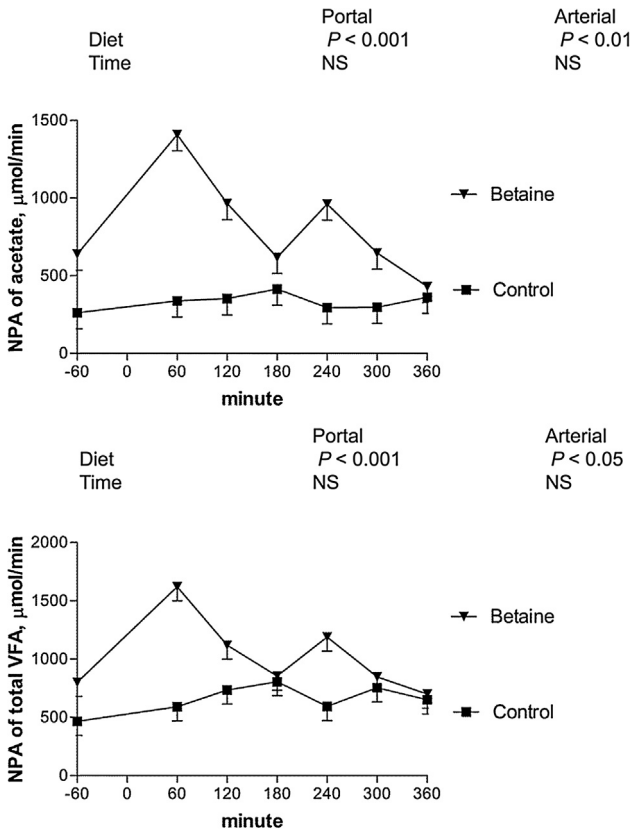

Fig. 2. Net portal absorption (NPA) of acetate and total volatile fatty acids (VFAs) in Iberian pigs fed control (■) or betaine (▲) diets. Values are means of four pigs. SEM: acetate = 76; total VFA = 86. NS: Not significant, $P > 0.10$.

Table 4
Mean values of bacterial groups abundance and proportion of bacterial groups relative to total bacteria determined by quantitative PCR in Iberian pigs ($n = 4$/diet) after feeding a diet supplemented or not with betaine (5 g/kg w/w).

	Control	Betaine	SEM	*P-value*
log *CFU equivalents/g fresh matter*				
Total bacteria	10.3	10.4	0.14	0.742
Lactobacillus	8.63	9.23	0.806	0.325
Bifidobacterium	6.96	7.77	0.925	0.251
Enterobacteriaceae	8.73	8.76	0.352	0.896
Bacteroides	8.68	8.81	0.427	0.666
Clostridium cluster I	6.68	6.61	0.651	0.885
Clostridium cluster IV	8.63	8.58	0.191	0.729
Clostridium cluster XIV	9.53	9.72	0.273	0.342
% of bacterial groups				
Lactobacillus	7.24	7.56	4.354	0.919
Bifidobacterium	0.10	4.22	3.646	0.148
Enterobacteriaceae	2.38	2.90	1.846	0.722
Bacteroides	1.59	1.82	0.533	0.611
Clostridium cluster I	0.0002	0.0007	0.00063	0.287
Clostridium cluster IV	2.20	1.85	1.005	0.640
Clostridium cluster XIV	19.5	23.5	4.98	0.470

In previous experiments, betaine increased ileal VFA and diaminopimelic acid (constituent of bacterial cell wall) concentrations in 9 kg BW barrows (Ratriyanto et al., 2009) although the results regarding the effect on nutrient digestibility were conflicting. However, it should be pointed out that, by means of an *in vitro* batch culture method utilizing frozen feces as inocula, we have shown that betaine modulates large intestine fermentation depending upon type of substrate of fermentation (Pardo et al., 2020).

The higher level of VFA observed in portal compared to arterial blood was expected as portal blood reflects the production of VFA in the gastrointestinal tract not consumed by colonocytes before uptake by the liver and later by the peripheral tissues (den Besten et al., 2013).

Acetate proportion in portal blood for control diet was 0.70, which is similar to growing Iberian pigs fed acorns (0.78; Lachica et al., 2019) and Large White pigs fed fiber rich diets (0.60; Rérat et al., 1987). Addition of betaine notably increased acetate proportion in portal blood (0.90). The mixture of individual VFA present in the arterial blood was almost exclusively composed of acetic acid (0.89 and 0.97 of total VFA for control and betaine, respectively), indirectly indicating a large uptake of propionate, butyrate, isobutyrate and valerate by the liver as previously noticed (Theil et al., 2016). Furthermore, a considerable fraction of acetic acid was taken up by the liver as well in the present experiment as shown by the reduction in acetate concentration in arterial relative to portal blood. Nevertheless, only by using hepatic balances measuring inputs (portal vein and artery) and outputs (hepatic vein), the hepatic uptake of VFA can be calculated (Rérat et al., 1987; Theil et al., 2016). Acetate portal proportion was 0.70–0.90 for control and betaine, respectively, which is within the range of values reported in the literature (Michel and Rérat, 1998; Agyekum et al., 2016). As expected portal proportions of propionate and butyrate were much lower (0.15 and 0.04 across treatments, respectively for propionate and butyrate), within the range of values previously reported (Agyekum et al., 2016; Lachica et al., 2019).

The NPA of total VFA of Iberian pigs fed the control diet was within the range of values reported in the literature for growing pigs fed diets with elevated level of corn or potato starch (335–1482 µmol/min; van der Meulen et al., 1997), diets with assorted dietary fiber sources (550–783 µmol/min; Theil et al., 2011) or diets with starch differing in rate of digestion (500–1350 µmol/min; Regmi et al., 2011). However, lower NPA of VFA have been reported in 34 kg BW Iberian pigs fed 600 g of acorns (400 µmol/min; Lachica et al., 2019).

As expected, acetate had the greatest NPA for both diets among VFA followed by propionate and butyrate. No differences in pre- and postprandial NPA of individual VFA were found similarly to portal and arterial concentrations. Yen et al. (1991) speculated that the explanation for a lack of postprandial increase in NPA of acetate could be a low endogenous (acetate released from portal drained viscera as a result of oxidative metabolism of mobilized fatty acids) contribution of acetate to portal blood and/or high postprandial intestine-tissue utilization for fat synthesis of absorbed acetate.

Betaine increased NPA of total VFA and hence the amount of VFA available for hepatic uptake. This augmented NPA of total VFA was due to increased NPA of acetate at the expense of NPA of propionate. On the other hand, betaine decreased the ratio of NPA of propionate to NPA of total VFA compared to control (0.40 and 0.16 for control and betaine diet, respectively). The ratios NPA of acetate and propionate to NPA of total VFA for the control diet was similar to those reported in growing crossbred pigs fed a corn soybean meal diet (0.42 and 0.38 for acetate and propionate, respectively; Yen et al., 1991) and Iberian growing pigs fed acorns (0.67 and 0.47 for acetate and propionate, respectively; Lachica et al., 2019). Supplementation with 30% distillers' dried grains with solubles of a corn and soybean meal diet fed to growing pigs (23 kg BW) increased the ratio of acetate to total VFA (0.36 and 0.85 for control and high fiber diet, respectively; Agyekum et al., 2016). The NPA of acetate, propionate and butyrate were positive, indicating net uptake from the gastrointestinal tract. Microbiota metabolizes branched chain amino acids and proline from undigested dietary and endogenous proteins (Macfarlane et al., 1992) arriving to the large intestine producing branched chain VFA (isobutyrate from valine, isovalerate from leucine and valerate from proline).

An increased concentration of these minor VFA is associated therefore with protein fermentative processes in the hindgut.

The VFAs are a source of energy for other tissues (Roberfroid, 2007). The absorbed VFAs are metabolized in ceco-colonic epithelial cells, which use butyrate as energy source, hepatocytes that metabolize propionate and butyrate for gluconeogenesis, and muscle cells that oxidize acetate (Roberfroid, 2007). Although a fraction of acetic acid arriving in portal vein was taken up by the liver, acetate concentration in arterial blood was considerably elevated especially in betaine pigs, suggesting that betaine increased energy availability in peripheral tissues. The VFA contribution to the maintenance energy requirements depends on diet composition, animal weight, meals per day or length of preprandial fasting, among others. For instance, energy from VFA accounted for 16% of ME for maintenance when heavy pigs (200 kg BW) were fed low fiber diets and 40% of ME for maintenance when a high fiber diet was used (Serena et al., 2009). The VFA absorbed could account for 0.20–0.27 of energy requirements for maintenance in the current study (15.4–16.1% NDF) in contrast with a contribution of 0.11 in 34 kg BW Iberian pigs fed 600 g of acorns (4.2% NDF; Lachica et al., 2019), in agreement with Serena et al. (2009), which may indicate a greater VFA production in the hindgut when pigs are fed diets with greater NDF content.

The production of VFA is determined by a number of factors, including the number and types of microorganisms present in the colon (Roberfroid, 2007). Therefore, composition and metabolic activity of microbiota may shed light regarding differences in VFA portal appearance in the present experiment. The mechanism behind the selective effect of betaine requires further investigation. Interestingly, a bran rich diet (betaine source) increased bacterial taxa of colonic microbiota that transformed betaine into other amino acid derived betaines with potential health benefits (Koistinen et al., 2019). Nevertheless, when evaluating the abundance of total bacteria and selected bacterial groups in pig feces in the present experiment, no differences were observed between control and treated pigs, suggesting that betaine supplementation only affected minor groups. Although associations between differential bacterial species and VFA production have been reported (Xiong et al., 2020) in growing-finishing pigs, betaine enhanced fermentation but did not affect the abundance of ruminal microbiota *in vitro* (Mahmood et al., 2020). It is possible that bacterial activity, not the amount of specific bacterial groups, was affected by betaine in the present experiment. Furthermore, fecal microbiota is reflective of large intestine populations (Williams et al., 2001) but not identical. In consequence, the fecal abundance of the specific bacterial groups studied in the present work was not related to the differences in NPA of VFA elicited by betaine.

Although our results support that betaine supplementation enhanced NPA of volatile fatty acids in growing Iberian pigs, care should be taken because of the low number of pigs used.

In conclusion, betaine increased net portal absorption of VFA and subsequently energy availability for liver and peripheral tissues in Iberian pigs without changing abundance of total bacteria and selected bacterial groups in feces.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2021.100197.

Ethics approval

The study protocol was approved by the Bioethical Committee of the National Research Council (CSIC), Spain.

Data and model availability statement

None of the data were deposited in an official repository. The data are available upon sensible request.

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Declaration of interest

No conflict of interest exists.

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