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ORIGINAL ARTICLE

Individual differences in behaviour and gut bacteria are associated in collared peccary (Mammalia, Tayassuidae)

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Abstract

Aims: We tested the hypothesis that the behaviour of an individual is associated with the diversity of its gut bacteria, using the collared peccary (*Pecari tajacu*) as a model.

Methods and Results: In all, 24 adult male collared peccaries received either low- ($n = 12$) or high-fibre diet ($n = 12$) to induce contrasting gut fermentation profiles. They were submitted to three short-term challenges, allowing us to rate the animals in a coping-style dimension named 'calmness'. At the end of the experimental period, we collected samples of peccaries' forestomach contents to characterize bacterial diversity. We found a significant positive association between individual 'calmness' z-scores and the bacterial evenness index in gut bacteria (and a similar trend with the Simpson's diversity index), suggesting a more homogeneous bacterial community of calmer individuals. We also found a positive association between fibres digestibility and gut bacterial diversity in the peccaries' forestomach, but no effect of the dietary fibre level.

Conclusions: Gut bacteria evenness increases with 'calmness' z-scores, suggesting a more homogeneous bacterial community of calmer individuals, compared with the more heterogeneous of the most distressed ones. Our results also suggest associations between the digestibility of ADF with the gut bacterial diversity indices and with the relative abundance of the *Actinobacteria* phylum.

Significance and Impact of the Study: Our data showed that the hosts' individual behavioural differences are potentially aligned with gut bacterial diversity. The behaviour–microbiota link is correlated with host feed efficiency and, ultimately, may have implications for animal health and welfare of farm animals.

Introduction

Herbivores rely on their ability to digest plant complex molecules, especially polysaccharides, to fulfil their nutritional requirements in energy and nutrients. They are, however, unable to produce enzymes to hydrolyse complex carbohydrates such as cellulose, hemicellulose and sometimes even starch. Thus, animals eating large

amounts of forage in their diets evolved to host synergetic microbial communities in their gastrointestinal tract, and the gene pool of these communities extends the digestive abilities of the host beyond its own enzymes. In mammals, for example, sections of the gastrointestinal tract either in a form of a forestomach or a voluminous hindgut, increased retention of digesta and hence facilitating exposure to gut microbiota (Dehority 2002; Stevens and Hume 2004).

Recent studies have highlighted the relationship between animal behaviour and the diversity of gut microbiota through the bidirectional communication between the central nervous system and the enteric nervous system, named the microbiota–gut–brain axis (see review by Kraimi *et al.* 2019). Stress and dietary diversity, for instance, can affect microbial diversity/activity and, consequently, the efficacy of the digestive processes (Cryan and Dinan 2012; Beck and Gregorini 2020). Studies have shown that individual behavioural differences in coping with stress have a strong impact on feed efficiency: more excitable individuals may be less efficient than non-excitable ones, as verified in beef cattle (Llonch *et al.* 2016; Neave *et al.* 2018). The domestication process and subsequent artificial selection for production traits focused on the selection of individuals with calmer behavioural traits (e.g. Rauw *et al.* 2017), which resulted in livestock with higher performance under production conditions than those with excitable behaviour (e.g. Bruno *et al.* 2016). It is also likely that selecting for higher average daily gains and feed efficiency has modified not only the animals themselves but also shaped their gut microbiota.

Individual differences in behaviour may be assessed by different approaches, such as personality, behavioural syndrome, temperament and coping style (see review by Finkemeier *et al.* 2018). In this study, we adopted the coping-style approach (Koolhaas *et al.* 2010), a sub-aspect of the personality concept (Finkemeier *et al.* 2018), to investigate whether interindividual behavioural differences and gut microbiota diversity are associated in collared peccaries (*Pecari tajacu*). This species is a prominent player in food security in the Amazon region (Nogueira *et al.* 2010), and its farming is strongly recommended as an efficient alternative strategy for meat production and subsistence in this region (Nogueira and Nogueira-Filho 2011). As its domestication process is still in a very early phase, with only few decades of selective breeding, the collared peccary is an interesting model to test the hypothesis that host behaviour is associated with gut bacterial diversity, owing to the high variability still noted between individuals (Nogueira *et al.* 2015; Borges *et al.* 2020).

Although a non-ruminant, the collared peccary has a forestomach composed of one fermentative pouch with two blind sacs (Langer 1978, 1979). The ingested feed undergoes anaerobic fermentation by the forestomach microbial community, which comprises bacteria (Lochmiller *et al.* 1989), protozoa (Carl and Brown 1983) and archaea (Oliveira *et al.* 2009), resulting in the fermentation end-products (short-chain fatty acids (SCFAs)—mainly acetate, propionate and butyrate) in concentrations similar to the ones found in the rumen of cattle and sheep (Sowls 1997). This fermentation allows the species to digest dietary fibre (neutral detergent fibre—NDF and acid detergent fibre—ADF) with relatively high efficiency (>50%), almost comparable with ruminants (Comizzoli *et al.* 1997; Nogueira-Filho 2005; Nogueira-Filho *et al.* 2018). Additionally, collared peccaries show great behavioural variation in reactions towards humans (Nogueira *et al.* 2015), and individual coping style is correlated with the digestibility of ADF in this species (Borges *et al.* 2020). Here we expanded this approach to evaluate the effects of coping style as well as dietary fibre level on the bacterial diversity in the forestomach contents of collared peccary.

As the species digests fibre efficiently, we expected the foregut bacterial composition of collared peccary to be more similar to the foregut bacteria found in ruminants than in the hindgut of pigs. Given that both stress (e.g. Duffy *et al.* 2018) and different levels of dietary fibre (e.g. Petri *et al.* 2013; Henderson *et al.* 2015) affect the gut microbial-community diversity and its products, we predicted that the diversity of the bacterial community in the forestomach contents of collared peccary would be linked with both the individuals' coping style and dietary fibre level. In turn, we expected that the concentrations of SCFAs and the pH in forestomach contents of collared peccaries would be linked to dietary fibre levels in the diets rather than to individual behavioural differences. This is expected because diet influences gut bacterial population, and the inclusion of readily fermentable carbohydrates promotes changes in the rumen microbiota, which causes changes in the concentrations of SCFAs as well as lower pH in the rumen (Hoover 1986; Goad *et al.* 1998; Fernando *et al.* 2010; Henderson *et al.* 2015). Age causes an increment in microbiota colonization, as has been reported in ruminants, pigs and wild horses (Rey *et al.* 2014; Niu *et al.* 2015; Metcalf *et al.* 2017); furthermore, dry-matter intake levels have a strong influence on the passage rate of digesta and fibre digestibility by the collared peccary (Nogueira-Filho *et al.* 2018) and can change gut microbiota populations in ruminants (Faria and Huber 1984; Hoover 1986). Therefore, we predicted that the bacterial-community diversity indices in collared

peccaries' forestomach would be linked both to their age and dry-matter intake. Additionally, we expected associations between the bacterial-community diversity indices in collared peccaries' forestomach and the digestibility of ADF.

Materials and methods

Animals, housing conditions and procedures

In all, 24 adult male collared peccaries, with an initial body weight of 21·6 ± 0·7 kg and average age of 2·50 years (SE = 0·05), ranging from 2·0 to 3·5 years, acquired from a commercial peccary farm, were used in digestion trials (Borges *et al.* 2020). Collared peccaries were maintained in the individual 11·3 m^2 (7·5 m × 1·5 m) pens of the Laboratory of Neotropical Animal Nutrition, Universidade Estadual de Santa Cruz, Brazil (14°47′39·8″S, 39°10′27·7″W). Each pen was divided into two sections: one covered area of 3·0 m^2—named the metabolism pen—had a wooden lattice suspended floor that allowed the faeces and urine to be separated, and an additional area, comprised of a partially sheltered section and a 'solarium' section, which allowed unobstructed exposure to natural sunlight, had a cement floor, as previously described by Borges *et al.* (2017).

Each experimental trial lasted for 56 days, and consisted of 30 days of habituation to the experimental conditions, 20 days for adaptation to the experimental diets, 5 days for digestion trials and behavioural assessment at different times. During the habituation period, animals received the same diet as was furnished on the commercial farm, composed of corn grain, soybean meal, grass hay, and seasonal fruits, such as banana, papaya and jack fruit mixed with mineral salt, resulting in a diet with 140 g kg^{-1} of crude protein and 14·5 MJ kg^{-1} of digestible energy on a dry-matter basis, following Borges *et al.* (2017) while water was available *ad libitum*. Thereafter, we allowed 20 days for adaptation to the experimental diets differing in fibre content, as explained below, and 5 days of the digestion trial.

At slaughter, which occurred after the threat test and weighing on the morning immediately following the end of the digestion trials, we collected forestomach content samples. As only six pens were available, the procedures were repeated four times ('periods') until all 24 animals had gone through the experimental procedures. Considering the experiment over time, we used temperature and humidity records from a nearby weather station, to quantify changes in gut bacterial data in response to the changing temperature–humidity index. The temperature–humidity index was calculated using the equation:

$$\text{Temperature} - \text{humidity index} = 9/5t + 32 - 11/2\,(1-h)\,(9/5t - 26),$$

where t is the maximum daily temperature in °C, and h is the minimum daily relative humidity (Ravagnolo and Misztal 2002).

The Ethics Committee for Animal Use of the Universidade Estadual de Santa Cruz approved all treatments and handling procedures adopted in this study (Protocol # 0102012), which was in full compliance with national legislation (authorization for experiments on captive wild animals, #1/29/2001/00022-7 by the Brazilian government's environmental agency—IBAMA).

Assessment coping style

Borges *et al.* (2020) assessed the interindividual differences in coping style by both the 'trait rating'—following the methods described by Feaver *et al.* (1986) and Wemelsfelder *et al.* (2000)—and the standard ethological record also named the 'behavioural coding' approach (*sensus* Vazire *et al.* 2007) of behaviours potentially indicative of stress (BPIS). Briefly, the trait ratings of peccaries were judged during three short challenge tests: following the protocols described by Nogueira *et al.* (2015): (1) novel-environment test: immediately after being released inside the metabolism pen—on the first day of the habituation period; (2) novel object test: sudden introduction of two coconuts into the metabolism pen—this procedure occurred 15 days after the novel environment test and (3) threat test: the keeper presents the capture-net just before the animal was transferred from the pens at the end of the digestive trial on the 56th day. Each animal's reactions were video-recorded using a digital camcorder (JVC, model GZHD500; Tokyo, Japan), fixed on a tripod and placed in front of the pen's chain-link door. Three judges rated the individual's reactions over the three challenge tests based on the video footage recorded during each test, following the qualitative behaviour assessment—QBA approach procedures (Wemelsfelder *et al.* 2000). QBA is based on 12 previously validated adjectives to assess relatively positive and negative emotional states of the collared peccary (Nogueira *et al.* 2015). Results indicated cross-time and context stability in closely correlated 'relaxed', 'quiet' and 'satisfied' responses, which were combined to yield *z*-score ratings of a coping-style dimension named 'calmness' that ranged from −1·0 to 2·6.

The standard ethological record data collection occurred twice a day for 30 days of the habituation period to the experimental conditions. The keeper was standing 0·5 m in front of the pen door and, using the same digital camcorder as described above, video-recorded each peccary for 10 min before he entered the

pen to furnish feedstuffs and clean up the pen. Two agonistic behaviours were considered as BPIS: 'tooth clacking' and 'whirling'. 'Tooth clacking' was described as an explosive series of 'clacks', made by rapid movements of the mandible, whereas 'whirling' occurs when the animal, with its mouth open, rapidly spun to face the animal keeper (Byers and Bekoff 1981). Two observers, who were blind to the experimental treatments during video-analysis, scored the total number of BPIS events exhibited by each individual on video-recorded images using the all-occurrences method (Altmann 1974) and the software CowLog 3.0.2 (Hänninen and Pastell 2009), and determined the frequencies of BPIS per h, which ranged from 0 to 11·9 events per hour of observation. The 'calmness' *z*-scores and the frequencies of BPIS per h were negatively correlated ($r_{\text{Spearman}} = -0{\cdot}45$, $P = 0{\cdot}03$, $n = 24$) (Borges *et al.* 2020).

Experimental diets

Briefly, the procedures of Borges *et al.* (2020) for the digestive trials were as follows. During both the adaptation to the experimental diets and digestion trial periods, the animals were fed one of the two experimental diets contrasting in fibre content (Table 1) in a completely randomized design. The experimental diets were formulated to meet the nutritional requirements of collared peccaries (Borges *et al.* 2017). In each group of six animals, three peccaries received the low-fibre diet while the three others received the diet with high-fibre levels. Diets were offered *ad libitum* twice a day at 08:00 and 17:00 h, and peccaries had *ad libitum* access to food until the next meal was offered (Borges *et al.* 2020), and after 20 days of adaptation to the diets (Nogueira-Filho 2005), peccaries were maintained in the metabolism pens, and faecal collection was carried out for five consecutive days. All feed samples, refusals (if any) and voided faeces were collected twice a day, at feeding times.

The diets provided contrasting levels of dietary fibre (low-fibre diet *vs* high-fibre diet) by varying the proportions of ground corn, soybean meal, Tifton hay (*Cynodon* sp.) and guava fruit (*Psidium guajava*) (Table 1). All ingredients were roughly mixed. The chemical composition of the leftovers did not differ from the diets offered, indicating that the animals did not select the diet ingredients (Borges *et al.* 2020). The proportions of NDF (546·8 g kg^{-1} of DM) and of ADF (268·9 g kg^{-1} of DM) in the high-fibre diet were 94·6 and 89·4%, respectively, well above the maximum recommendable levels of 281 g NDF kg^{-1} of DM and 142 g ADF kg^{-1} of DM for collared peccary (Nogueira-Filho 2005). Such dietary fibre levels resulted in different daily mean intakes of NDF and ADF (Borges *et al.* 2020).

Table 1 Ingredients (g kg^{-1} as-is) and analysed nutrient composition (g kg^{-1} of DM unless otherwise mentioned) of the experimental diets (as fed basis) among collared peccaries (N = 24)*

Ingredients	Proportions Low fibre	High fibre
Corn	836	397
Soybean meal	98	97
Tifton (*Cynodon dactylon*) hay	1	50
Guava fruit	60	451
Mineralized salt	4	4
Mineral premix	0·5	0·5
Vitamin premix	0·5	0·5
Analysed nutrient composition	Low fibre	High fibre
Dry matter	880·2	590·6
Crude protein (N × 6·25)	120·0	122·6
NDF	193·9	546·8
ADF	90·8	268·9
DE (MJ kg^{-1} of DM)	15·1	13·3

Mineral premix: iron, 180 g; copper, 20 g; cobalt, 4 g; manganese 80 g; zinc 140 g; iodine, 4 g.
Vitamin Premix: Vitamin A, 1 200 000 IU; Vitamin D3, 1 500 000 IU; Vitamin E, 1 500 000 IU; Vitamin B1, 2 g; Vitamin B2, 4 g; Vitamin B6, 4 g; Vitamin B12, 20 000 g; calcium pantothenate, 15 g; biotin, 0·10 g; Vitamin K3, 3 g; folic acid, 0.6 g; nicotinic acid, 20 g; Zn bacitracin, 20 g; methionine, 100 g; L-lysine, 300 g; choline chlorine, 100 g, butylated hydroxytoluene (BHT), 10 g; selenium 0·10 g.
*Modified from Borges *et al.* (2020). NDF, neutral detergent fibre; ADF, acid detergent fibre.

Forestomach content sample analyses

At the end of the digestive trials, the peccaries were euthanized 3 h after their last meal. This duration had been established in a pilot study and allowed the researchers to collect enough material for the proposed analyses. Immediately after euthanasia, we opened the abdominal cavity of the animals and used ligatures to bind the oesophagus at the cardia and the forestomach at the junction with the glandular stomach. Forestomach content samples (from both blind sacs and main chamber) were collected and homogenized by animal. Thereafter, we measured the pH in the forestomach content samples using a unipolar electrode (MS Tecnopon Instrumentação®, Piracicaba, SP, Brazil). After the pH determination, the samples were snap-frozen in liquid nitrogen and preserved at −80°C until further bacterial DNA extraction and SCFAs analyses.

DNA was extracted in the Laboratório de Nutrição Animal, UESC, from the forestomach contents (0·25 g) of each animal, individually, using the Powerfecal™ DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA) following the manufacturer's instructions. Thereafter, samples were sent to the University of Liège for DNA sequencing. The PCR amplification of the V1–V3 regions of the 16S rRNA and library preparation were performed

with the following primers: forward (5′-GAGAGTTT GATYMTGGCTCAG-3′) and reverse (5′-ACCGCGGCT GCTGGCAC-3′) and Illumina overhand adapters, used before by Moula *et al.* (2018), Fastrès *et al.* (2019) and Cong *et al.* (2020). Thermocycling conditions consisted of a denaturation step at 94°C for 15 min followed by 25 cycles of 94°C for 40 s, 56°C for 40 s, 72°C for 1 min and a final elongation step of 7 min at 72°C. These amplifications were performed on an Ep Master system gradient apparatus (Eppendorf, Hamburg, Germany).

Each PCR product was purified with the Agencourt AMPure XP beads kit (Beckman Coulter, Pasadena, CA) and submitted to a second PCR round for indexing, using the Nextera XT index primers 1 and 2 (Illumina, San Diego, CA). After purification, PCR products were quantified using Quant-IT PicoGreen (ThermoFisher Scientific, Waltham, MA) and diluted to 10 ng μl^{-1}. A final quantification, by qPCR, of each sample in the library was performed using the KAPA SYBR® FAST qPCR Kit (KapaBiosystems, Wilmington, DE) before normalization, pooling and sequencing on a MiSeq sequencer using v3 reagents (2 × 300, Illumina). Processing of sequence reads was done using, respectively, MOTHUR software package ver. 1.35 (Schloss *et al.* 2009) and the UCHIME algorithm (Edgar *et al.* 2011) for alignment and clustering and chimera detection. Datasets were subsampled at 10 000 read per sample to assess bacterial diversity in peccaries' forestomach contents, using Simpson's reciprocal diversity (diversity), evenness (evenness) indices and Chao1 richness estimator (Chao1) at genus level using MOTHUR. Subsampling size was determined by analysing rarefaction sampling curves with MOTHUR. Good's coverage of the subsampled datasets was calculated with MOTHUR, which reached mean values of 95% for species level and 99% for genus level.

The Simpson's reciprocal diversity index is a bacterial diversity measurement based on operational taxonomic units (OTUs), which ranges between 0 and 100—the greater the Simpsons' diversity index the higher the diversity. The Chao1 richness estimator and evenness index are complementary factors when measuring diversity. The Chao1 richness estimator is an abundance estimator based on the number of species represented by just one or two individuals, that is, this index shows the importance of rare OTUs (Chao 1984). The more species present in a sample, the 'richer' the sample. In turn, the evenness index compares the uniformity of the population size of each species of bacteria present in the sample; the higher the evenness, the more similar the population sizes of the bacterial species (Kim *et al.* 2017).

The gut bacterial phylogenetic composition was summarized at phylum level based on relative abundance (sum of sequences per taxon divided by total sequences). Unclassified taxa within a given taxonomic level were not pooled together, but rather were pooled according to their closest classifiable parent (e.g. unclassified phylum belonging to *Bacteria*). Good's coverage estimator was used as a measure of sampling effort for each sample. All sample raw reads were deposited at the Genbank and are available under bio-project number PRJNA665146.

The SCFAs (acetate, propionate and butyrate) analyses were performed at the Laboratório de Nutrição Animal, Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, São Paulo. In this laboratory, after thawing, the forestomach content samples (2 ml) were centrifuged (RC 5B PLUS; Sorval, Wilmington, DE) at 13 500 ***g*** for 40 min at 4°C, and 800 µl of the supernatant was collected in microtubes, to which 100 µl of 2-ethyl-butyric acid was added (internal standard MW = 116·16; Sigma Chemie Gmbh, Steinheim, Germany) and 200 µl formic acid (85%). Subsequently, a 1 µl aliquot was injected in a gas chromatograph (GC 2014 Shimadzu, Tokyo, Japan) equipped with FID and with the column GP 10% SP-1200/1 H_3PO_4 80/100 Chromosorb WAW (Cat. no. 11965, 6′ × 1/8″ stainless steel; Supelco, Bellefonte, PA). Column temperature (isothermal) was at 120°C, injector at 160°C and detector at 190°C. The carrier gas was He at 25 ml min^{-1}. Detector hydrogen and synthetic air were kept at 40 and 400 ml min^{-1}, respectively. A calibration curve was prepared with standards of known concentrations (acetate 99·5%, CAS 64-19-97; propionate 99%, CAS 04-09-79; and butyrate 98·7%, CAS 107-92-6; Chem Service, West Chester, PA).

Statistical analysis

To test our prediction of links between the individual differences in behaviour and dietary fibre level and the bacterial community in the forestomach of collared peccary, we used general linear models (GLMs) to compare the Simpson's diversity and evenness indices, and Chao1 richness estimator in the bacterial community at genus level, as well as the relative abundance of bacteria at phylum level in the forestomach of collared peccary (one model per measure). The models included the diet (low-fibre diet *vs* high-fibre diet) as fixed factor and the 'calmness' *z*-scores or the frequency of BPIS per h as co-variables, followed by linear regression analysis when appropriate. We also included in the GLM models the 'period' as a random effect, as it was not possible to collect data from all 24 animals at the same time. Finally, we included the age and dry-matter intake as co-variables in the GLM models. As there were no significant effects of age ($P > 0{\cdot}14$, Table S1) and dry-matter intake ($P > 0{\cdot}11$, Table S1), we excluded these co-variables from the final GLM models. Only the phyla that constituted

1·0% or more of the relative abundance were statistically evaluated. We used the same statistical models to compare the concentrations of SCFAs (acetate, propionate and butyrate) and pH of the forestomach content samples. Residuals of the models were tested for normality of errors and homogeneity of variance. Square root transformations were used for Simpson's diversity index and Chao1 richness estimator, acetate, propionate and butyrate concentrations, whereas logarithmic transformations were used for the frequency of BPIS per h and the relative abundances of the bacteria phyla Bacteroidetes, Actinobacteria and Proteobacteria to meet the assumptions of the approach. We applied a Bonferroni correction for multiple comparisons at $\alpha = 0{\cdot}005$ (0·05/10 comparisons) for GLM analyses. Based on GLM results, we used Pearson correlation tests to check possible associations between the Simpson's diversity and evenness indices, and Chao1 richness estimator, as well as evaluating these diversity indices and the gut bacteria phyla in association with the digestibility of ADF, after proper transformations when necessary. We used *t*-tests to compare temperature–humidity index means of the four experimental 'periods'. Thereafter, we tested the association between the temperature–humidity index and the gut bacteria phyla using Pearson correlation tests as well. We also used Pearson correlation tests to check the association between the gut bacteria phyla and the concentrations of SCFAs. Finally, nonparametric tests were used to analyse the pH in the forestomach contents samples, as they did not meet assumptions even after transformations. We set the significance level at $\alpha = 0{\cdot}05$ for Pearson correlation tests and nonparametric tests. We used the software Minitab 19.1 (Minitab Inc., State College, PA) for all analyses.

Results

The V1–V3 gene pyrosequencing carried out on forestomach contents of collared peccaries generated 14 076 OTUs (an OTU was defined as a read sharing ≥97% nucleotide sequence identity). After performing Good's coverage test, we verified that the coverage range of 23 samples was 0·92–0·98 (Table S1), a sufficient sample for all but one individual (collared peccary #14).

There was a significant effect of 'calmness' *z*-scores on the evenness index ($F_{1,\ 20} = 12{\cdot}99$, $P = 0{\cdot}002$, $n = 23$, Table 2). The greater the 'calmness' *z*-scores, the higher the evenness index of the bacterial community at genus level in forestomach contents of collared peccaries (Simpson's evenness diversity index = 0·06 + 0·01 'calmness' *z*-scores, $F_{1,\ 21} = 14{\cdot}18$, $R^2 = 0{\cdot}40$, $P = 0{\cdot}001$, $n = 23$, Fig. 1). This effect remained significant, even excluding the individual with the highest 'confidence' *z*-score (Simpson's evenness diversity index = 0·06 + 0·01 'calmness' *z*-scores, $F_{1,\ 20} = 12{\cdot}30$, $R^2 = 0{\cdot}38$, $P = 0{\cdot}002$, $n = 22$). There was also a trend of association between the 'calmness' *z*-scores and the Simpson's diversity index ($F_{1,\ 20} = 7{\cdot}64$, $P = 0{\cdot}012$, $n = 23$, Table 2); the greater the 'calmness' *z*-scores, the higher the diversity index of the bacterial community at genus level in forestomach contents of collared peccaries while there was no effect of 'calmness' *z*-scores on the Chao1 richness estimator (Table 2). There were no effects of the frequencies of BPIS per h; dietary fibre level or period on the Simpson's diversity and evenness indices and Chao1 richness estimator (Table 2).

The Simpson's diversity index was positively correlated with the evenness index ($r_{Pearson} = 0{\cdot}85$, $P < 0{\cdot}001$, $n = 23$) and with the Chao1 richness estimator ($r_{Pearson} = 0{\cdot}80$, $P < 0{\cdot}001$, $n = 23$). Additionally, there was a trend of correlation between the evenness indices with the Chao1 richness estimator ($r_{Pearson} = 0{\cdot}40$, $P = 0{\cdot}058$, $n = 23$). In turn, the digestibility of ADF was correlated with the Simpson's diversity index ($r_{Pearson} = 0{\cdot}43$, $P = 0{\cdot}039$, $n = 23$) while there were trends of correlations between the digestibility of ADF with the evenness index ($r_{Pearson} = 0{\cdot}37$, $P = 0{\cdot}085$) and Chao1 richness estimator ($r_{Pearson} = 0{\cdot}36$, $P = 0{\cdot}095$).

We detected a total of 15 phyla, 25 classes, 49 orders, 32 families and 162 genera. The most predominant

Table 2 Means (standard errors) of the Simpson's diversity (diversity) and evenness (evenness) indices, and the Chao richness estimator (Chao1) of the bacterial community at genus level in the forestomach contents of collared peccaries fed low- ($n = 12$) or high-fibre diet ($n = 11$) and relationships ($F_{1,\ 20}$ and *P* values) with coping-style metrics ('calmness' *z*-scores and frequencies of BPIS per h) and dietary fibre level (diet)

	Low fibre	High fibre	'Calmness' *z*-scores	Diet	Period	Frequencies of BPIS per h	Diet	Period
Diversity	14·56 (1·57)	14·20 (2·98)	$F_{1,17} = 6{\cdot}28$ ($P = 0{\cdot}023$)	$F_{1,17} = 0{\cdot}00$ ($P = 0{\cdot}973$)	$F_{3,17} = 0{\cdot}09$ ($P = 0{\cdot}963$)	$F_{1,17} = 1{\cdot}67$ ($P = 0{\cdot}214$)	$F_{1,17} = 0{\cdot}02$ ($P = 0{\cdot}878$)	$F_{3,17} = 0{\cdot}20$ ($P = 0{\cdot}895$)
Evenness	0·06 (0·01)	0·07 (0·01)	$F_{1,17} = 11{\cdot}49$ ($P = 0{\cdot}003$)	$F_{1,17} = 0{\cdot}49$ ($P = 0{\cdot}494$)	$F_{3,17} = 0{\cdot}59$ ($P = 0{\cdot}628$)	$F_{1,17} = 2{\cdot}00$ ($P = 0{\cdot}175$)	$F_{1,17} = 0{\cdot}67$ ($P = 0{\cdot}426$)	$F_{3,17} = 0{\cdot}51$ ($P = 0{\cdot}680$)
Chao1	405·70 (42·90)	400·03 (45·10)	$F_{1,17} = 1{\cdot}50$ ($P = 0{\cdot}238$)	$F_{1,17} = 0{\cdot}16$ ($P = 0{\cdot}693$)	$F_{3,17} = 0{\cdot}61$ ($P = 0{\cdot}615$)	$F_{1,17} = 0{\cdot}38$ ($P = 0{\cdot}544$)	$F_{1,17} = 0{\cdot}08$ ($P = 0{\cdot}777$)	$F_{3,17} = 0{\cdot}68$ ($P = 0{\cdot}579$)

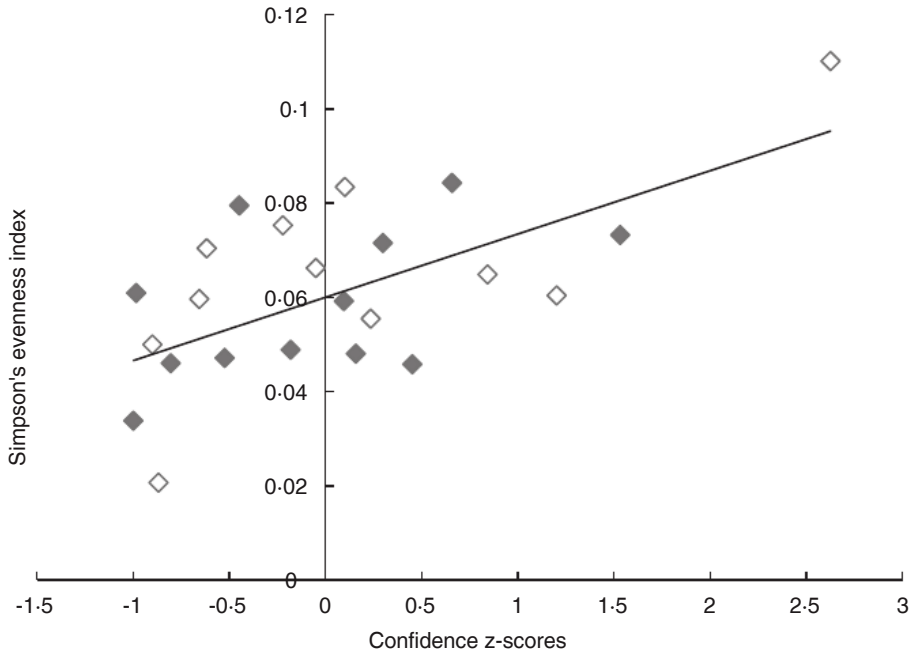

Figure 1 Relationships between Simpson's evenness index of bacteria present in the forestomach of collared peccaries fed low- (◆; $n = 12$) or high-fibre diet (◇; $n = 11$) and the individual's 'confidence' z-scores, according to the equation: Simpson's evenness index = 0·06 + 0·01 'confidence' z-score ($F_{1, 21} = 14{\cdot}18$, $R^2 = 0{\cdot}40$, $P = 0{\cdot}001$, $n = 23$).

bacterial phyla identified in forestomach contents were Bacteroidetes (mean = 63·5%, SE = 7·0) and Firmicutes (mean = 27·0%, SE = 5·5), respectively; followed by *Actinobacteria* (mean = 4·5%, SE = 1·7) and Proteobacteria (mean = 1·1%, SE = 0·4). There were another 10 phyla present in forestomach contents, but at lower relative abundances that together represented a mean of 1·4% (SE = 0·9) of OTUs while unclassified *Bacteria* represented 2·4% (SE = 0·5) as well. Examining each sample composition at the phylum level revealed noticeable differences between individual collared peccaries (Table 3).

The phylum *Bacteroidetes* was composed mainly of the genus *Prevotella* (mean = 62·5%, SE = 7·2, min = 0·1% max = 94·2%) (Table S2). The phylum Firmicutes was composed mainly of the genus *Kandleria* (mean = 5·3%, SE = 3·7, min = 0·0% max = 83·3%) while the phylum Actinobacteria was composed mainly of the genus *Bifidobacterium* (mean = 2·1%, SE = 1·0, min = 0·0% max = 19·8%) (Table S2). The complete information on bacteria present in the forestomach of collared peccary is found in the supplementary data (Table S2). There was only a near-significant positive effect of the 'calmness' z-scores on the relative abundance of the Actinobacteria phylum ($F_{1, 17} = 9{\cdot}48$, $P = 0{\cdot}007$, $n = 23$, Table 3). There were significant effects of the 'period' on the relative abundances of the phyla Firmicutes and Actinobacteria

Table 3 Means (standard errors) of the relative abundances (%) of bacteria at phylum level in the forestomach contents of collared peccaries fed low- ($n = 12$) or high-fibre diet ($n = 11$) and relationships ($F_{1, 20}$ and P values) with coping-style metrics ('calmness' z-scores and frequencies of BPIS per h) and dietary fibre level (Diet)

Phylum	Low fibre	High fibre	'Calmness' z-scores	Diet	Period	Frequencies of BPIS per h	Diet	Period
Bacteroidetes	65·08 (9·34)	61·80 (11·10)	$F_{1,17} = 0{\cdot}07$ ($P = 0{\cdot}792$)	$F_{1,17} = 0{\cdot}80$ ($P = 0{\cdot}382$)	$F_{3,17} = 6{\cdot}12$ ($P = 0{\cdot}005$)	$F_{1,17} = 1{\cdot}13$ ($P = 0{\cdot}304$)	$F_{1,17} = 0{\cdot}70$ ($P = 0{\cdot}415$)	$F_{3,17} = 6{\cdot}95$ ($P = 0{\cdot}012$)
Firmicutes	23·23 (6·13)	31·16 (9·47)	$F_{1,17} = 1{\cdot}72$ ($P = 0{\cdot}208$)	$F_{1,17} = 0{\cdot}50$ ($P = 0{\cdot}487$)	$F_{3,17} = 9{\cdot}28$ ($P = 0{\cdot}001$)	$F_{1,17} = 2{\cdot}46$ ($P = 0{\cdot}135$)	$F_{1,17} = 0{\cdot}63$ ($P = 0{\cdot}438$)	$F_{3,17} = 9{\cdot}74$ ($P = 0{\cdot}001$)
Actinobacteria	5·68 (2·59)	3·25 (2·16)	$F_{1,17} = 9{\cdot}48$ ($P = 0{\cdot}007$)	$F_{1,17} = 4{\cdot}68$ ($P = 0{\cdot}045$)	$F_{3,17} = 41{\cdot}95$ ($P < 0{\cdot}001$)	$F_{1,17} = 2{\cdot}02$ ($P = 0{\cdot}173$)	$F_{1,17} = 2{\cdot}63$ ($P = 0{\cdot}124$)	$F_{3,17} = 29{\cdot}21$ ($P < 0{\cdot}001$)
Proteobacteria	1·34 (0·61)	0·93 (0·44)	$F_{1,17} = 2{\cdot}42$ ($P = 0{\cdot}138$)	$F_{1,17} = 0{\cdot}51$ ($P = 0{\cdot}483$)	$F_{3,17} = 0{\cdot}83$ ($P = 0{\cdot}496$)	$F_{1,17} = 1{\cdot}10$ ($P = 0{\cdot}309$)	$F_{1,17} = 0{\cdot}34$ ($P = 0{\cdot}569$)	$F_{3,17} = 1{\cdot}32$ ($P = 0{\cdot}300$)

and a trend towards an effect of the 'period' on the relative abundance of Bacteroidetes (Table 3). There were positive correlations between the temperature–humidity index and the relative abundances of Firmicutes and Actinobacteria ($r_{Pearson} = 0{\cdot}70$, $P < 0{\cdot}001$, $n = 23$; $r_{Pearson} = 0{\cdot}83$, $P < 0{\cdot}001$, $n = 23$, respectively) while there was a negative correlation between the temperature–humidity index and the relative abundance of Bacteroidetes ($r_{Pearson} = -0{\cdot}85$, $P < 0{\cdot}001$, $n = 23$). There were no effects of the frequencies of BPIS per h and dietary fibre level on the relative abundances of the bacteria phyla (Table 3).

The temperature–humidity index mean recorded in the fourth 'period' was higher (t value $< -4{\cdot}45$; $P < 0{\cdot}047$) than the temperature–humidity index means of the other three previous periods, which did not differ among each other (t value $< -6{\cdot}67$; $P > 0{\cdot}573$) (period 1: 70·4, SE = 0·2; period 2: 71·0, SE = 0·3; period 3: 72·7, SE = 2·3; period 4: 78·7, SE = 1·4). Thus, after excluding the data collected during the fourth period from the GLM models, we verified a significant positive effect of the 'calmness' z-scores on the relative abundance of the Actinobacteria phylum ($F_{1,\,12} = 13{\cdot}34$, $P = 0{\cdot}003$, $n = 17$) and near-significant positive effects of the 'calmness' z-scores on the relative abundance of Bacteroidetes ($F_{1,\,12} = 6{\cdot}24$, $P = 0{\cdot}028$, $n = 17$) and Firmicutes phyla ($F_{1,\,12} = 9{\cdot}03$, $P = 0{\cdot}011$, $n = 17$). There was a positive correlation between the digestibility of ADF and the relative abundance of the Actinobacteria phylum ($r_{Pearson} = 0{\cdot}60$, $P = 0{\cdot}011$, $n = 17$), which ranged from 0·0 to 24·5% (Table S2). Additionally, there was a trend towards a negative correlation between the digestibility of ADF and the relative abundance of the Bacteroidetes phylum ($r_{Pearson} = -0{\cdot}43$, $P = 0{\cdot}091$, $n = 17$) as well as a trend towards a positive correlation between the digestibility of ADF and the relative abundance of the Firmicutes phylum ($r_{Pearson} = 0{\cdot}41$, $P = 0{\cdot}098$, $n = 17$). No correlation was found between the digestibility of ADF and the relative abundance of the Proteobacteria phylum ($r_{Pearson} = 0{\cdot}25$, $P = 0{\cdot}328$, $n = 17$).

The most abundant SCFA in forestomach contents was acetate, followed by propionate and butyrate (Table 4). We found a trend towards a higher concentration of acetate in the forestomach contents of collared peccaries fed the high-fibre diet compared to the ones fed the low-fibre diet ($F_{1,\,18} = 7{\cdot}37$, $P = 0{\cdot}014$, $n = 24$, Table 4). There was, however, no significant effect of dietary fibre level on the concentrations of propionate and butyrate in forestomach contents (Table 4). There were also no effects of coping-style metrics and 'period' on the concentrations of SCFAs in forestomach contents (Table 4). Moreover, there were no correlations between the gut bacteria phyla and the concentrations of SCFAs (Table 5).

The dietary fibre level affected the pH in forestomach contents of collared peccary (high-fibre diet: median = 5·3, min = 4·9, max = 7·3; low-fibre diet: median = 5·0, min = 4·7, max = 5·3; Mann–Whitney $U = 193{\cdot}50$, $P = 0{\cdot}013$, $n = 24$). There were, however, no effects of either coping-style metrics ('calmness' z-scores:

Table 5 Pearson's coefficient of correlations between the relative abundances (%) of bacteria at phylum level and the concentrations of short-chain fatty acids (SCFAs: acetate, propionate and butyrate) in the forestomach contents of collared peccaries fed low- (n = 12) or high-fibre diet (n = 11)

Phylum	SCFAs	$r_{Pearson}$	s
Bacteroidetes	Acetate	−0·19	0·377
	Propionate	−0·10	0·708
	Butyrate	−0·19	0·399
Firmicutes	Acetate	0·06	0·786
	Propionate	−0·02	0·918
	Butyrate	0·23	0·289
Actinobacteria	Acetate	−0·10	0·638
	Propionate	0·11	0·605
	Butyrate	0·19	0·383
Proteobacteria	Acetate	0·21	0·330
	Propionate	0·39	0·065
	Butyrate	−0·16	0·453

Table 4 Means (standard errors) of concentrations of short-chain fatty acids (SCFAs: acetate, propionate and butyrate—μmol ml^{-1}) in the forestomach contents of collared peccaries fed low- (n = 12) or high-fibre diet (n = 12) and relationships ($F_{1,21}$ and P values) with coping-style metrics ('calmness' z-scores and frequencies of BPIS per h) and dietary fibre level (Diet)

SCFAs	Low fibre	High fibre	'Calmness' z-scores	Diet	Period	Frequencies of BPIS per h	Diet	Period
Acetate	71·5 (0·9)	76·7 (1·5)	$F_{1,18} = 0{\cdot}18$ ($P = 0{\cdot}678$)	$F_{1,18} = 7{\cdot}37$ ($P = 0{\cdot}014$)	$F_{3,18} = 0{\cdot}09$ ($P = 0{\cdot}965$)	$F_{1,18} = 1{\cdot}36$ ($P = 0{\cdot}258$)	$F_{1,18} = 7{\cdot}98$ ($P = 0{\cdot}011$)	$F_{3,18} = 0{\cdot}13$ ($P = 0{\cdot}941$)
Propionate	15·2 (0·6)	13·6 (0·9)	$F_{1,18} = 0{\cdot}38$ ($P = 0{\cdot}545$)	$F_{1,18} = 3{\cdot}02$ ($P = 0{\cdot}099$)	$F_{3,18} = 1{\cdot}14$ ($P = 0{\cdot}360$)	$F_{1,18} = 0{\cdot}27$ ($P = 0{\cdot}611$)	$F_{1,18} = 3{\cdot}26$ ($P = 0{\cdot}088$)	$F_{3,18} = 1{\cdot}63$ ($P = 0{\cdot}217$)
Butyrate	8·0 (0·8)	7·0 (0·3)	$F_{1,18} = 0{\cdot}53$ ($P = 0{\cdot}476$)	$F_{1,18} = 0{\cdot}91$ ($P = 0{\cdot}352$)	$F_{3,18} = 2{\cdot}19$ ($P = 0{\cdot}124$)	$F_{1,18} = 1{\cdot}98$ ($P = 0{\cdot}176$)	$F_{1,18} = 1{\cdot}24$ ($P = 0{\cdot}280$)	$F_{3,18} = 2{\cdot}03$ ($P = 0{\cdot}145$)

$r_{Spearman} = 0{\cdot}10$, $P = 0{\cdot}64$, $n = 24$; frequency of BPIS per h: $r_{Spearman} = -0{\cdot}30$, $P = 0{\cdot}16$, $n = 24$) or period (Kruskal–Wallis H-value = 1·93, $P = 0{\cdot}59$, $n = 24$) on the pH in forestomach.

Discussion

Our findings support the assumption that the bacterial composition in the forestomach contents of collared peccary would have more similarities with the bacteria found in the foregut of ruminants than in the hindgut of pigs. The majority of bacteria, found in forestomach contents of collared peccary fed diets composed of 50% of grains or more, were from the genus *Prevotella* (Phylum—Bacteroidetes). *Prevotella* is also the most abundant bacterial genus in ruminants fed a grain-rich diet (Henderson *et al*. 2015), such as cows (Stevenson and Weimer 2007; Jami and Mizrahi 2012), sheep (Bekele *et al*. 2010), goats (Metzler-Zebeli *et al*. 2013) and domesticated Sika deer (*Cervus nippon*, Li *et al*. 2013). Conversely, a combination of the genera *Clostridium* and *Lactobacillus* or *Clostridium* and *Eubacterium* (Phylum—Firmicutes) prevails in the hindgut microbiota of domestic and wild pigs, respectively (Ushida *et al*. 2016).

These differences can be attributed to the place where fermentation takes place. In collared peccaries and ruminants, fermentation occurs in the foregut (pre-gastric fermentation), while in pigs, fermentation occurs in the hindgut (post-gastric fermentation). Thus, in ruminants and collared peccaries the microbial community uses the diet before the host, resulting in different microbial ecosystems in comparison to pigs and thus explaining differences in gut bacterial community between these species.

The targeted V1–V3 variable regions of the 16S rRNA gene were effective in highlighting the existence of a potential link between host individuals' behavioural traits and the evenness of the bacterial community in forestomach contents of collared peccary. The diversity metrics used to describe the bacterial community in forestomach contents of collared peccary take into account two aspects of a community: the number of different organisms in a sample and the abundance of each one. Simpson's diversity index is an estimator of species richness with more weight on species evenness, whereas the Chao1 richness estimator gives more weight to the low-abundance species (Kim *et al*. 2017). Therefore, the diversity metrics' characteristics may explain the relationships between the 'confidence' z-scores and not only Simpson's diversity and evenness indices but also the lack of relationship with the Chao1 richness estimator verified here. Additionally, as commented before, the evenness and species richness are complementary factors of diversity and, with the increase of evenness and species richness, in general, diversity increases as well (Kim *et al*. 2017). Our results show that the gut bacterial evenness increases with 'calmness' z-scores, suggesting a more homogeneous bacterial community of calmer individuals, compared with the more heterogeneous community of the most distressed ones.

The correlation results point to associations between the bacterial diversity metrics in peccaries' forestomach and the digestibility of ADF, which may be associated with the increase in the digestibility of ADF by calmer individuals (Borges *et al*. 2020). Intuitively, higher bacterial diversity, along with the phenotypical behavioural variability of the collared peccary (Nogueira *et al*. 2015), is good for a non-domesticated species such as the collared peccary, as it allows the species to thrive in a variety of habitats, eating different kinds of food, from prickly pear cacti (*Opuntia* sp.) in semi-arid regions (Sowls 1997) to fruits and leaves in tropical rain forests (Kiltie 1981). These traits are also possibly linked with the ease of adaptation of farmed collared peccaries to a variety of diets, from domestic pig formulae to different agricultural by-products with high dietary fibre levels (Nogueira-Filho *et al*. 2018; Andrade *et al*. 2020). In domestic ruminants submitted to selection for higher average daily gains and feed efficiency (Rauw *et al*. 2017), the lower microbiome diversity is linked to higher feed efficiency, as shown by Shabat *et al*. (2016). Therefore, the precise reasons for the associations between bacterial diversity metrics in peccaries' forestomach and the digestibility of ADF, and their causal direction, need to be determined in further research, which may indicate whether they support the hypothesis that individual differences in behaviour indeed play a role in the digestibility of dietary fibre.

In our pilot study, we were not able to collect a representative forestomach sample, which contained both liquid and solid fractions, via oesophageal tubing as described by Paz *et al*. (2016), and thus it was necessary to euthanize the animals for the proper collection of samples. To avoid the loss of animals, this kind of study could be done using hindgut fermenters. In this context, Ushida *et al*. (2016) and Metcalf *et al*. (2017) studied the effects of domestication on gut microbiota of pigs and horses. Although Ushida *et al*. (2016) found differences in the relative abundance of some bacterial genera between wild and domestic pigs, the authors did not evaluate the relationship between individual differences in behaviour and the gut microbiota. In turn, Metcalf *et al*. (2017) verified a more diverse gut bacterial community for Przewalski's horses (*Equus ferus przewalskii*) in comparison with domestic horses, which were linked to differences in their diets. Metcalf *et al*. (2017) verified lower interindividual variation in the gut microbiota of Przewalski's horses than in domestic horses. These authors,

however, did not evaluate the relationship between individual differences in behaviour with the gut microbiota.

Alternatively, we recommend studying the relationship between hosts' individual behavioural differences with the diversity of their gut bacteria, using the capybara (*Hydrochoerus hydrochaeris*) or the paca (*Cuniculus paca*) as models. Just like peccaries, both species are under domestication in Neotropical countries (Nogueira and Nogueira-Filho 2011; Jones *et al.* 2019) and thus may show high behavioural variability between individuals, as already verified in paca (Nogueira *et al.* 2021). These rodents show caecotrophic behaviour—the ingestion of special faeces enriched in microbial protein by colonic separation mechanisms (Nogueira-Filho *et al.* 2013; Aldrigui *et al.* 2018a, 2018b). Additionally, capybaras and pacas usually excrete soft faeces or 'caecotrophs' which consists of caecal material (Mendes *et al.* 2000; Aldrigui *et al.* 2018a, 2018b). Therefore, the collection of 'caecotroph' samples would allow proper collection of gut microbial communities from the caecum segment of both species, potentially confirming a causal relationship between microbiota–gut–brain axis and feed efficiency.

The effect of the 'confidence' *z*-scores on the relative abundances of bacteria at the phylum level found in the forestomach contents of collared peccary has most likely been hampered by the mean temperature–humidity indexes recorded in the four experimental 'periods'. The temperature–humidity index is a measure used to quantify the effect of heat stress on domestic animals and its effect on milk yield or pig growth (e.g. Ravagnolo *et al.* 2000 and Zumbach *et al.* 2008, respectively). In the present study, we verified that the higher the temperature–humidity index, the higher the relative abundances of the Firmicutes and Actinobacteria phyla, together with a decrease in the relative abundance of the Bacteroidetes phylum. Bacteria belonging to Firmicutes and Actinobacteria phyla play a critical role in fermentation of cellulose and hemicellulose (Fernando *et al.* 2007). In particular, Actinobacteria participate in the initiation process of complex substrate degradation (Makki *et al.* 2018). Our results corroborate this role, as the relative abundance of the Actinobacteria phylum was positively correlated with the digestibility of ADF. These results point to an influence of ambient temperature and humidity on the bacterial composition in the forestomach of collared peccary: the higher the temperature–humidity index, the greater the ability of collared peccary to degrade dietary fibre. This trait is potentially connected with the good productive performance of the species' on high-fibre diets in regions with high ambient temperature and humidity (Andrade *et al.* 2020). After excluding the data collected in the fourth period from the GLM models, we verified that the higher the 'calmness' *z*-scores, the higher the relative abundance of the *Actinobacteria* phylum. There was an increase of ~16% in the digestibility of ADF by the individuals with negative *z*-scores, compared the ones with positive *z*-scores (Borges *et al.* 2020). Thus, our results point to a higher feed efficiency among calmer individuals, based on their ability to digest dietary fibre compared to the distressed ones. However, studies are still needed to strengthen a causal relationship between individual differences in behaviour, gut bacteria changes and digestive efficiency.

In the present study, only the 'subjective' ratings were able to detect core individual differences in the behaviour of collared peccaries linked to the bacterial community in the forestomach of collared peccary. However, Borges *et al.* (2020) reported that both the 'confidence' *z*-scores and frequency of BPIS per h were aligned with the digestibility of ADF. However, the 'subjective' ratings approach is a form of integrative qualitative assessment of an animal's conditions based on observing the dynamic whole individual, taking into account the context (Cooper and Wemelsfelder 2020). In turn, the 'behavioural coding' approach consists only of measuring the length or number of certain behavioural patterns. Such differences in both approaches to access individual differences in behaviour thus possibly explain these apparently contradictory results between Borges *et al.* (2020) and the ones obtained in this study. Scharen *et al.* (2018) also failed to determine a link between cows' behaviour and their rumen microbiota diversity, using the standard ethological record of feeding intake pattern (e.g. meal duration and frequency). Therefore, our results support the affirmation by Vazire *et al.* (2007) that 'trait ratings' are more reliable in detecting consistencies in an individual's behavioural traits than is 'behavioural coding'.

Contrary to our expectations, there was no relationship between dietary fibre level and the diversity of the bacterial community in forestomach contents of collared peccaries. Usually, low- and high-fibre diets require different levels of diversity of the microbiota for appropriate digestion (e.g. Petri *et al.* 2013; Henderson *et al.* 2015). Although there were no significant effects of dry-matter intake on the bacterial diversity indices, as commented before, the low dry-matter intake by collared peccaries fed the high-fibre diet compared to the ones fed the low-fibre diet resulted in lower daily intakes of digestible energy (DE) and digestible protein (DP) (Borges *et al.* 2020). In cattle, it has been reported that increased consumption of dietary energy and protein sources leads to an increase in microbial action and consequently fibre fermentation in the rumen (Faria and Huber 1984; Hoover 1986). Taking this into account, the low dietary intake of energy and protein sources in collared peccaries

may not have allowed the full potential growth of bacteria in the forestomach when these animals are fed the high-fibre diet. This would result in the lack of effect caused by dietary fibre level on the diversity of the bacterial community found in forestomach contents, as found here.

The mean concentrations of SCFAs determined in forestomach contents of collared peccary are in the concentration ranges previously determined for this species (Lochmiller *et al.* 1989; Sowls 1997). The production of acetate and propionate in forestomach contents of collared peccary could be associated with the higher relative abundance of *Prevotella* species, which are positively correlated with acetate and propionate concentrations in ruminants (Xue *et al.* 2016). However, we found no association between bacterial phyla and the concentration of the SCFAs. Additionally, the concentrations of SCFAs determined here differed from the concentration ranges determined in ruminants. Moreover, the mean concentration of acetate was higher while the mean concentrations of propionate and butyrate were lower in the forestomach contents of collared peccaries than the concentration ranges determined in the rumen of cattle and sheep (Sowls 1997). Besides bacterial profile, however, the production of SCFAs is dependent on many other variables (e.g. bacterial metabolism and competition for substrates) (Hernandez-Sanabria *et al.* 2010), and may come from the fermentation of plant cell wall compounds by protozoa (Carl and Brown 1983) and archaea (Oliveira *et al.* 2009), which are part of the microbial community of the collared peccary's forestomach. Therefore, further studies on the whole consortium of anaerobic microbes in the forestomach of collared peccary and their relationship with interindividual behavioural differences and diet are still required.

Nevertheless, the relatively high concentrations of SCFAs in the forestomach contents of collared peccary point to considerable microbial fermentation and exploitation of dietary fibre by this species. As we expected, the concentrations of these fermentation end-products in the forestomach are linked to dietary fibre level rather than to individuals' coping style. There was a trend towards a higher concentration of acetate in the forestomach of collared peccaries fed the high-fibre diet than the ones fed the low-fibre diet. Comparable results have been reported for cattle (Sung *et al.* 2015; Lee *et al.* 2016).

The pH range in forestomach contents of collared peccaries determined in this study is similar to the ones previously reported for this species (Lochmiller *et al.* 1989; Oliveira *et al.* 2009). The relatively low mean pH in the forestomach contents in this study was probably due to an inevitable mixture of fluids between the forestomach and the glandular stomach, as suggested by Lochmiller *et al.* (1989). This backflow from glandular stomach to forestomach probably explains the lack of difference in the pH of the forestomach contents of collared peccaries fed low- and high-fibre diets. Another possible explanation for this relatively acid pH in forestomach contents of collared peccary may be that this species does not chew the cud. The pH in the foregut of ruminants is maintained in the range from 6·0 to 7·0, due to the copious salivary secretion containing bicarbonate and phosphate salts, which helps buffer the acids produced by fermentation (Allen 1997). Despite that, as expected, collared peccaries fed a grain-rich diet showed lower pH than ones fed a high-fibre diet, as verified in ruminants (Hoover 1986; Goad *et al.* 1998). The lack of effect of age on the analysed variables probably occurred because we only used mature individuals with complete microbiota colonization.

In conclusion, our results showed that the forestomach of collared peccary harboured a combination of bacteria that can degrade fibre. Gut bacteria evenness increases with 'calmness' *z*-scores, suggesting a more homogeneous bacterial community in calmer individuals, compared with the more heterogeneous community in the most distressed ones. Our results also suggest that the digestibility of ADF is associated with the gut bacterial diversity indices and with the relative abundance of the Actinobacteria phylum. Therefore, this study provides a novel insight into how the behaviour-microbiota link can influence the gut bacterial community and potentially impact host digestion of nutrients. It suggests that, ultimately, feed efficiency may have implications for the husbandry, health and welfare of farm animals.

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Conflict of Interest

The authors declare no conflicts of interest.

Authors' contribution

Pedro L. G. Cairo; Vanessa S. Altino: Investigation, Writing—Original draft preparation, Writing—Reviewing and Editing. Sérgio Nogueira-Filho; Jérôme Bindelle; Selene S. C. Nogueira; Marc Vandenheede: Conceptualization, Methodology, Formal analysis, Writing—Reviewing and Editing. Bernard Taminiau: Genetic relatedness analysis, Writing—Reviewing and Editing. Martine Schroyen, Georges Daube, Eduardo Gross: Methodology; Writing—Reviewing and Editing.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Relationships (*F* and *P* values) between the Simpson's diversity (Diversity) and evenness (Evenness) indices, and the Chao richness estimator (Chao1), the relative abundances (%) of bacteria at phylum level of the bacterial community at genus level in the forestomach contents of collared peccaries fed low- ($n = 12$) or high-fiber diet ($n = 11$) with coping-style metrics ('calmness' *z*-scores or frequencies of BPIS h^{-1}), dietary fiber level (Diet), period, age and dry matter intake (DMI—g $kg^{-0.75}$).

Table S2. Descriptive statistics (means, standard errors—SE, minimum and maximum values) of the relative abundance (%) of bacteria found in the forestomach contents of collared peccaries ($n = 23$) at phylum, class, order, family, and genus levels.