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RESEARCH ARTICLE

Feeding a Low-protein Maternal Diet Affects Qinghai Bamei Piglet Jejunal Structure and Microbial Function Response

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Abstract: This experiment investigated the impacts of feeding a maternal low-CP concentration diet having iso-essential amino acids on newborn suckling piglet's intestinal microbial composition and function. Forty randomly selected purebred Bamei sows were divided into two groups and fed a low dietary CP (12%, LP) or a normal CP (14%, CON) diet, respectively, but formulated to contain similar (iso-) essential amino acid concentrations per current recommendations. At 21 days, 12 piglets were randomly selected from each treatment and euthanized with jejunum content samples collected. The 16S rRNA gene sequencing was combined as an integrated approach for evaluating the functional impact of maternal CP concentrations on piglet intestinal microbiome. Even though piglets demonstrated similar 0 to 21 d ADG among treatments, the jejunum relative weight, villus width, crypt depth and muscular thickness were increased (P<0.05), while villus height, and villus height/crypt depth were reduced (P<0.05) for the material LP compared to the maternal fed CON diet. Maternal CP concentrations can modify the intestinal microbial composition of Bamei suckling piglets. The relative abundances of the bacterial species *Escherichia-Shigella*, *Actinobacillus*, *Clostridium*_sensu_stricto_1, *Veillonella*, and *Turicibacter* were increased (P<0.05) in the maternal LP fed diet compared with the maternal fed CON diet microbiota metabolites. Overall, LP diet contributed to improve piglet intestinal histomorphology, microbial composition and function.

Keywords: *Qinghai Bamei piglet, Low-protein maternal diet, Intestinal histomorphology, 16S rRNA, Bioinformatics*

Düşük Proteinli Maternal Diyet ile Besleme Qinghai Bamei Domuz Yavrularının Jejunal Yapısını ve Mikrobiyal Fonksiyon Yanıtını Etkiler

Öz: Bu çalışmada, izo-esansiyel amino asitlere sahip düşük CP konsantreli maternal bir diyetle beslenmenin, yeni doğmuş süt emen domuz yavrularının bağırsak mikrobiyal bileşimi ve işlevi üzerindeki etkileri araştırıldı. Rastgele seçilen kırk safkan Bamei domuzu iki gruba ayrıldı ve sırasıyla düşük CP (%12, LP) ve normal CP (%14, CON) diyetle beslendi. Ancak, her iki diyet de güncel tavsiyelere göre benzer (izo-) esansiyel amino asit konsantrasyonlarını içerecek şekilde formüle edildi. Her iki diyet grubundan 21. günde rastgele 12 domuz yavrusu seçildi, ötenazi yapıldı ve jejunum içerikleri toplandı. 16S rRNA gen sekans entegreli bir yaklaşım ile maternal CP konsantrasyonlarının domuz yavrularının bağırsak mikrobiyomu üzerindeki fonksiyonel etkisi değerlendirildi. Her iki diyet grubundaki domuz yavruları, 0 ile 21. günler arası benzer ADG göstermiş olsa da, CON diyetine kıyasla maternal LP diyeti ile beslenenlerde jejunum relatif ağırlığı, villus genişliği, kript derinliği ve kas kalınlığı artmış (P<0.05), villus yüksekliği ve villus yüksekliği/kript derinliği azalmıştı (P<0.05). Maternal CP konsantrasyonları, süt emen Bamei domuz yavrularının bağırsak mikrobiyal bileşimini değiştirebilir. Maternal CON diyetle beslenenlere kıyasla maternal LP ile beslenenlerde *Escherichia-Shigella*, *Actinobacillus*, *Clostridium*_sensu_stricto_1, *Veillonella* ve *Turicibacter* bakteri türlerinin relatif yoğunlukları artmıştı (P <0.05). Genel olarak, LP diyeti, domuz yavrularının bağırsak histomorfolojisinin, mikrobiyal bileşimin ve işlevinin iyileştirilmesine katkıda bulunmuştur.

Anahtar sözcükler: *Qinghai Bamei domuz yavrusu, Düşük proteinli maternal diyet, Bağırsak histomorfolojisi, 16S rRNA, Biyoinformatik*

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Introduction

The Bamei is a local swine breed in the Qinghai Province of the People's Republic of China. Even though Bamei is a slow growing breed, Bamei swines are known for their high meat quality and distinctive flavor [1,2]. The Qinghai plateau has used both natural and artificial selection practices for developing Bamei pigs that show a strong adaptability to the plateau, have high fat deposition, and good meat quality characteristics. However, Bamei's lower growth rate combined with the plateau's low feed quality/digestibility are important constraints limiting the Qinghai's growth potential of the Bamei swine industry [3].

The gastrointestinal tract's microbial ecosystem is dynamic and complex with the composition known to vary widely across healthy individuals [4]. In the human and animal gastrointestinal tract there is a large and diverse microbial community playing a vital role in host health [5], mucosal immunological environment maturation [6,7] and assisting with intestinal barrier integrity [8]. Over the last decade, numerous studies have reported that the intestinal microbiome composition plays an important role in regulating the metabolic health of both rodents and humans [9]. A recent study conducted on rodents suggests the major dietary factors regulating intestinal microbiome taxonomic composition are protein and carbohydrate intake [10].

The intestinal microbiome is in a continual state of flux and highly susceptible to numerous environmental factors, especially dietary nutrient supply. Reducing CP by 2 to 4 percentage units by adding crystalline amino acids (AA) to meet NRC (2012) nutrient recommendations has increased nitrogen utilization, reduced feed costs and nitrogen excretion, while promoting intestinal health and meat quality with similar growth performance [11,12]. Many studies demonstrate dietary CP concentrations versus CP source, have a greater impact on intestinal microbiota composition [13,14]. Previous studies have focused on changes in large intestinal microbiota, while ignoring the bacteria's role for the small intestine [15]. Moderate diet protein restriction may alter intestinal microbiota composition while improving adult pig ileal barrier function [16,17]. Chen reported that decreasing dietary CP concentration 3 % units reduced ileal *Streptococcus* spp., while increasing *Lactobacillus* spp. and *Bifidobacterium* spp. [18]. These ileal microbiota alterations improved intestinal stem cell proliferation and altered tight junction protein distribution resulting in similar intestinal barrier function. Therefore, feeding dietary LP concentrations has advanced while maintaining essential amino acid supply and has been applied to swine production. The purpose of this study was to explore the effects of low protein diet on the structure and function of intestinal microflora of Qinghai Bamei pigs, to lay a foundation for further exploration of the effects of maternal dietary intervention on jejunal microbiota composition and function to provide ideas for efficient breeding of Qinghai Bamei pigs.

Material and Methods

Ethical Approval

All procedures involving the use of animals were approved by the Animal Care Committee of Qinghai University, China (QHDX-17-02-12-06). Animal slaughtering was approved by the National Administration of Slaughtering and Quarantine regulations (Qinghai, China).

Animals and Diets

Forty (40) purebreds Huzhu Bamei well body condition (score 4) sows were sourced through the Qinghai Province Huzhu County Bamei Pig Seed Breeding Farm (Huzhu, China) having similar body weight (BW), health status, and 3 to 4 years of age being randomly assigned to one of two treatments (20/treatment). The LP treatment diet (12% CP) was balanced for the five EAA Lys, Met, Thr, Trp, and Val for their standardized ileal digestibility (SID) concentrations and then decreased CP by 2% compared to a control (CON; 14% CP) diet balanced for the same SID EAA according to Chinese feeding standards for a 90 kg heavy body conditioned sow. The complete diet composition is given in *Table 1*. After 5 d of facility and diet

Table 1. *Ingredient and nutrient composition of maternal diets (DM basis) containing 12% (LP) or 14% crude protein (CON). DM basis) %*

Items	Groups	
	LP	CON
Ingredient composition		
Corn	50.60	44.90
Soybean meal	4.50	9.80
Rapeseed meal	2.50	2.70
Wheat bran	37.78	38.14
Lys	0.34	0.20
Met	0.07	0.05
Thr	0.15	0.10
Trp	0.02	0.01
Val	0.04	0.10
4% premixb	4.00	4.00
Nutrient concentrations, calculated via formulation		
DE (MJ/kg) a	11.72	11.72
CPb	12.04	12.04
Lys	0.81	0.81
Met+Cys	0.33	0.33
Thr	0.35	0.35
Trp	0.08	0.08
Val	0.26	0.26
Total Ca	0.62	0.62
Total P	0.51	0.51
Solt	3.20	3.20

[a] DE=digestible energy; [b] CP=crude protein; [b] The premix during pregnancy provided the following per kilogram of diets: Vit. A: 3.52 kIU; Vit. E: 20 kIU; Vit. D_3: 0.76 kIU; Vit. K_3: 2.6 mg; Vit. B_2: 9.52 mg; Vit. B_3: 24 mg; Vit. B_5: 45 mg; Cu: 4 mg; Fe: 10 mg; Zn: 40 mg; Mn: 16 mg; Ca: 15 %; Total P: 1.8%; NaCl: 8%; Water: 10 %

acclimation, the sows were fed the assigned treatment diet while skipping one estrous cycle (21 days) during natural estrus and then mated. The newborn piglets were housed with their mothers prior to weaning with litter size, live birth %, birth weights, and diarrhea rates being published previously [19]. Throughout the study all the sows had ad libitum access to feed and fresh water.

Sample Collection

Randomly, 12 piglets were selected from each treatment group, fasted for 12-h, weighed, and euthanized with 50 mg/kg sodium pentobarbital on day 21 of age. The small intestine was ligated at the pylorus, duodenum, jejunum, and ileum and dissected. The ligated jejunum was weighed. The jejunal contents were sampled at approximately the half-way point of the jejunal length, placed into 1.5 mL sterile polypropylene tubes, and stored in liquid nitrogen until analyses were conducted for intestinal microbiome. An approximate 1.5 cm jejunal tissue sample was collected, washed, and placed in 4% paraformaldehyde for histomorphometric analysis at the same time.

Histomorphometric Analysis

Jejunal tissue samples fixed in 4% paraformaldehyde were embedded in paraffin (5 μm) and stained with HE (hematoxylin-eosin). In each jejunal section, 12 intact villi were randomly selected from each piglet. The jejunum villus height, villus width, crypt depth, and muscular layer thickness were measured using an image analysis system (Caseviewer 2.0 software, 3DHISTECH, Hungary).

gDNA Extraction, 16S rRNA Gene Sequencing and Microbial Function Prediction

The jejunal content samples were extracted to harvest total bacterial DNA using the PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. The DNA samples were stored at -80°C until outsourced for analyzing the 16S rRNA gene sequencing by BIOMARKER (Beijing, China). The 16S rRNA gene sequence (Illumina HiSeq 2500) was used to measure microbial diversity and bacterial community composition. The extracted DNA was used as a template and PCR was performed using barcode primers located on both sides of the V3-V4 hypervariable region of the bacterial 16S rRNA gene. The primer sequences used were: 338F: 5'-ACTCCTACGGGAGGCAGCA-3' and 806R: 5'-GGACTACHVGGGTWTCTAAT-3'. Amplification was performed for 30 cycles using a DNA thermal Cycler (Bio-Rad, Hercules, CA, USA). The first cycle was at 98°C for 2 min followed by 30 subsequent cycles of 98°C x 30 s, 50°C x 30 s, then 72°C x 1 min, and the last cycle at 72°C for 7 min.

Statistical Analyses

All data were checked for outliers before any statistical analyses were conducted. Data were either plotted or the box and whisker plots and the Shapiro Wilk Test were used to verify that the data were normally distributed ($P>0.15$). All data were subjected to least squares analysis of variance (ANOVA) for a completely random design (CRD; Steel and Torrie, 1980) having 2 treatments using SPSS 21 software (SPSS Inc., Chicago, IL, USA). Least squares means were separated using the Least Significant Difference (LSD) and significant was declared at $P<0.05$.

The OTU were rarified based on several metrics for alpha diversity analysis including OTU rank curves, rarefaction, and Shannon, along with Shannon, Chao1, Simpson, and ACE calculated indices. Principal Coordinates Analysis (PCoA) and unweighted pair group method with arithmetic mean (UPGMA) were performed using QIIME based weighted UniFrac distance for beta diversity analysis [20]. Finally, PICRUSt [21] was used to predict microbial function. Bacterial domains, phyla, and genera were compared using Wilcoxon rank-sum test, with the FDR adjusted P value <0.05 being considered as significantly different. Finally, Spearman's rank correlations among jejunal microbiome changes, histomorphometric, and shifted metabolome were calculated to examine functional impacts of material LP diet concentrations on the small intestinal microbiome.

Results

Piglet Performance

Piglet birth BW (day 0) was greater for sows fed LP compared with piglet birth BW for sows fed CON ($P>0.05$), while 21 d piglet BW tended ($P<0.05$) to be greater for piglets from sows fed LP compared with sows fed CON *(Table 2)*. However, these initial and final piglet BW differences did not affect piglet ADG, which was similar among both treatments ($P>0.05$).

Table 2. *Piglet body weight (BW) and average daily gain (ADG) when feeding maternal diets containing 12% (LP) or 14% crude protein (CON)*

Items		LP	CON	SDM	P-value
Piglet BW, kg	Day 0	0.90	0.88	0.02	0.020
	Day 21	3.85	3.78	0.09	0.067
	ADG, 0 - 21, g/d	135.8	134.0	1.38	<0.37

Jejunal Morphology

Intestinal HE staining demonstrated that piglets nursing sows fed a maternal LP diet demonstrated reduced (P<0.05) villus height and ratio of villus height to crypt depth, while jejunum relative weight, villus width, crypt depth, and muscle thickness were increased (P<0.05) compared with piglets from sows fed the maternal CON diet *(Table 3)*.

The Diversity and Composition of Jejunal Microbiota

The 16S RNA jejunal microbiota samples after data filtering, quality control, and low-confidence singletons removal resulted in an average of 42.718 reads being obtained for the 21 d samples. The Good's coverages exceeded 99% demonstrating excellent sequence accuracy and reproducibility *(Table 4)*. Of the 482 total OTU numbers, 452 OTU were detected in both groups. Based on the Shannon (P<0.001), and Simpson (P=0.001) indices piglets from the maternal fed LP diet demonstrated more diversity and greater evenness compared with piglets from the material fed CON diet. The Chaol (P=0.519) and Ace (P=0.435) indices were similar for piglets from the maternal fed LP compared with the maternal fed CON. Taxonomic analysis revealed the predominant phyla *Firmicutes* and *Proteobacteria* being 67.21% and 24.97%, respectively of total reads identifying 16 bacterial phyla *(Fig. 1-A)*. At the genus level, 232 genera were identified in the jejunal samples. The predominant genera were *Lactobacillus* (51.11%), *Escherichia*-Shigella (9.00%), *Actinobacillus* (7.41%), *Clostridium*_sensu_stricto_1 (5.60%), *Romboutsia* (4.35%), and *Buchnera* (3.54%), respectively *(Fig. 1-B)*. Furthermore, using a PCoA plot illustrated microbial community dissimilarity and revealed distinct structures between piglets from the maternal fed LP compared with maternal fed CON *(Fig. 1-C)*. The PCoA plot uses a weighted method for UniFrac similarity, which revealed PC1 and PC2 explained 55.61% and 13.98% of sample variation, respectively. Similarly, the jackknifed beta diversity and hierarchical clustering analysis via the Unweighted Pair-group Method with Arithmetic Mean (UPGMA) demonstrated that different piglets fed different maternal CP diets were clustered in their individual groups *(Fig. 1-D)*. In addition, piglets from maternal fed CON diets in the PCoA plot were clustered into two subgroups and UPGMA hierarchical clustering analysis, which was attributed to individual variations of jejunum microbiome profiles.

Differences in Jejunal Bacterial Community Composition

Relative phylum abundances of *Firmicutes, Proteobacteria, Bacteroidetes,* and unknown were > 1% for both treatments *(Table 5)*. Firmicutes relative abundance was decreased (P=0.002) and Proteobacteria (P=0.001) was increased for piglets from the maternal LP treatment compared with piglets from the sows fed maternal CON. Thirty-two (32) specific genera demonstrated relative abundances >0.1%. The relative bacterial community abundances of *Escherichia*-Shigella (P=0.050), *Actinobacillus* (P=0.050), *Clostridium*_sensu_stricto_1 (P=0.003), *Veillonella* (P=0.015), and *Turicibacter* (P=0.011) were higher, and *Lactobacillus* was lower (P<0.001) for piglets from the

Table 3. *Jejunum weight and tissue morphology by 21-day old suckling piglets when feeding maternal diets containing 12% (LP) or 14% crude protein (CON)*

Items	LP	CON	SDM	P-value
Jejunum weight, g	123.22	109.95	17.12	0.074
Jejunum relative weight, %	3.42	3.17	0.30	0.048
Villus height, μm	318.58	385.44	17.99	<0.001
Villus width, μm	96.44	83.43	3.62	<0.001
Crypt depth, μm	150.15	99.01	6.58	<0.001
Villus height: Cryptdepth	2.13	4.62	0.19	<0.001
Muscular thickness, μm	65.17	60.75	2.24	<0.001

Table 4. *Alpha diversity measures of bacterial communities by 21-day old suckling piglets when feeding maternal diets containing 12% (LP) or 14% crude protein (CON)*

Items	LP	CON	SDM	P-Value
Chao1	218.08	208.89	33.48	0.519
Ace	216.58	205.47	33.66	0.435
Shannon	2.72	1.67	0.68	<0.001
Simpson	0.16	0.45	0.13	0.001
Coverage	0.9996	0.9996	<0.001	0.898

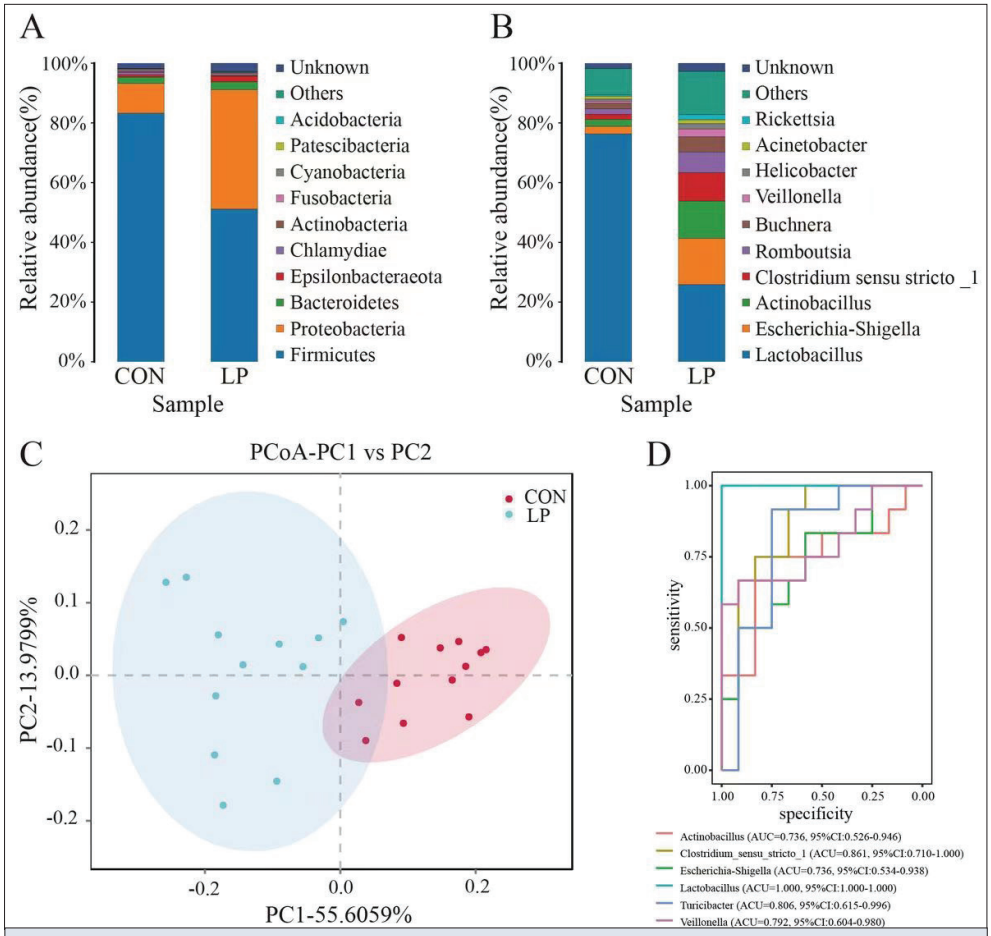

Fig 1. Classification of the bacterial community composition) by 21-day old suckling Bamei piglets when feeding maternal diets containing 12% (LP) or 14% crude protein (CON). **A**- Phylum level, **B**- genus level, **C**- PCoA plot, **D**- UPGMA tree. The relative abundance of the top 10 phylum and genus of jejunal microbiome composition profiles revealed by 16S rRNA sequencing (each color represents one bacterial). PCoA plot and UPGMA tree using the weighted unifrac similarity method

Table 5. *Phylum-level taxonomic composition of the jejunal bacterial communities by 21-day old suckling piglets when feeding maternal diets containing 12% (LP) or 14% crude protein (CON)*

Phylum	LP	CON	SDM	P-value
Firmicutes	0.51169	0.83253	0.17449	0.002
Proteobacteria	0.39987	0.09948	0.15060	0.001
Bacteroidetes	0.02626	0.02173	0.03188	0.299
Chlamydiae	0.00004	0.00804	0.01304	0.686
Epsilonbacteraeota	0.01906	0.00739	0.02340	0.166
Cyanobacteria	0.00210	0.00414	0.00565	0.773
Fusobacteria	0.00397	0.00372	0.00485	0.525
Actinobacteria	0.00452	0.00332	0.00593	0.356
Patescibacteria	0.00176	0.00111	0.00204	0.817
Acidobacteria	0.00110	0.00032	0.00140	0.840
Tenericutes	0.00070	0.00014	0.00112	0.544
Cloacimonetes	0.00009	0.00010	0.00035	0.544
Chloroflexi	0.00048	0.00007	0.00072	0.312
Verrucomicrobia	0.00008	0.00005	0.00020	0.356
Planctomycetes	0.00024	0.00002	0.00037	0.908
Gemmatimonadetes	0.00022	0.00002	0.00056	0.470
Unknown	0.02785	0.01781	0.02738	0.156

maternal fed LP treatment compared with piglets from the maternal fed CON treatment (genus level; *Table 6)*. The receiver operating characteristic curve (ROC) predicted different microorganisms for piglets from maternal fed LP compared to maternal fed CON piglets for inducing jejunal development. The area under the curve (AUC) judged via diagnosis test [22] that *Lactobacillus* is the most likely biomarker (0.9< AUC <1.0) for piglets from both treatments, while *Clostridium*_sensu_stricto_1 and *Turicibacter* are more likely biomarkers (0.8< AUC <0.9) for piglets from maternal fed LP sows.

Predicted Function of Jejunal Microbiota

The PICRUSt analyzed pathway compositions for evaluating jejunal bacterial community functional capacity is a functional-gene-count matrix. Second level KEGG (levels) metabolism pathway analysis via global and overview maps demonstrated that biosynthesis of other secondary metabolites was enriching amino acid, cofactors, and vitamins metabolism (P<0.05), while lipid and nucleotide metabolism were decreased (P<0.05) for piglets when maternal sows were fed LP diet compared with piglets from the maternal fed CON *(Fig. 2)*.

Table 6. *Genus-level taxonomic composition of the jejunal bacterial communities by 21-day old suckling piglets when feeding maternal diets containing 12% (LP) or 14% crude protein (CON)*

Genus	LP	CON	SDM	P-value
Lactobacillus	0.25881	0.76331	0.13670	<0.001
Escherichia-Shigella	0.15483	0.02514	0.12003	0.050
Actinobacillus	0.12509	0.02318	0.07921	0.050
Buchnera	0.05169	0.01920	0.05861	0.488
Romboutsia	0.06841	0.01856	0.06543	0.166
*Clostridium*_sensu_stricto_1	0.09503	0.01698	0.07304	0.003
Acinetobacter	0.01295	0.00957	0.01571	0.248
Prevotella_7	0.00384	0.01020	0.02064	0.436
Chlamydia	0.00004	0.00804	0.01298	0.686
Helicobacter	0.01813	0.00691	0.02292	0.094
Veillonella	0.02581	0.00659	0.01388	0.015
Turicibacter	0.00703	0.00440	0.01058	0.011
Rickettsia	0.01763	0.00407	0.01963	0.686
Uncultured_bacterium_f_Muribaculaceae	0.00853	0.00352	0.00993	0.326
Fusobacterium	0.00326	0.00329	0.00419	0.644
Pseudomonas	0.00922	0.00300	0.01422	0.106
Terrisporobacter	0.01388	0.00331	0.01267	0.299
Bacteroides	0.00514	0.00264	0.00618	0.184
Enterobacter	0.00117	0.00237	0.00358	0.603
Megasphaera	0.01073	0.00276	0.01537	0.386
Streptococcus	0.00261	0.00183	0.00164	0.149
Pasteurella	0.00642	0.00150	0.00635	0.194
Uncultured_bacterium_f_Lachnospiraceae	0.00161	0.00105	0.00270	0.795
Epulopiscium	0.00100	0.00116	0.00153	0.225
Citrobacter	0.00164	0.00093	0.00206	0.453
*Prevotellaceae*_UCG-001	0.00160	0.00064	0.00226	0.149
Lachnoclostridium	0.00174	0.00070	0.00185	0.100
Uncultured_bacterium_f_Clostridiales_vadinBB60_group	0.00295	0.00067	0.00352	0.260
Wolbachia	0.00205	0.00058	0.00233	0.624
Acidaminococcus	0.00419	0.00065	0.00800	0.386
Sutterella	0.00240	0.00023	0.00299	0.356
Others	0.05272	0.03520	0.01200	0.150
Unknown	0.02785	0.01781	0.02738	0.156

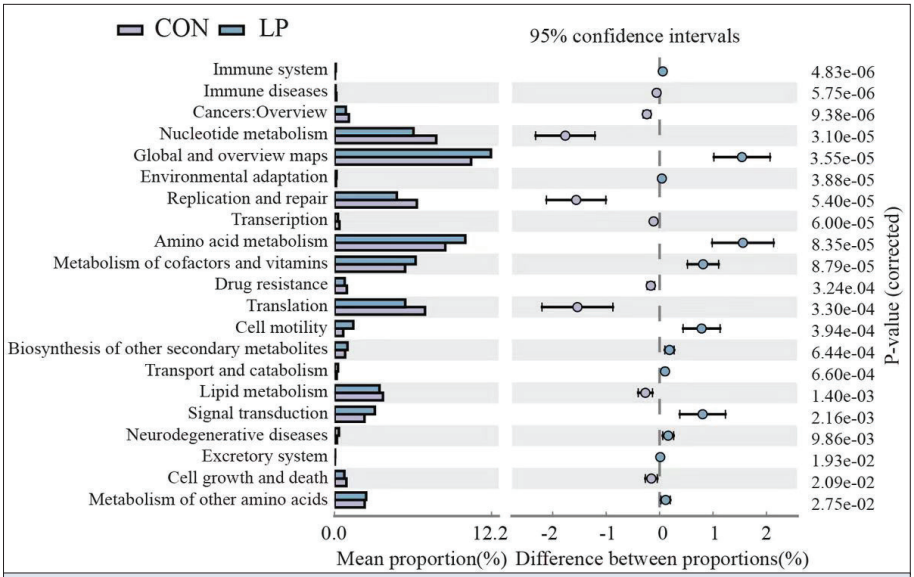

Fig 2. Predicted microbial functions using PICRUSt by 21-day old suckling piglets when feeding maternal diets containing 12% (LP) or 14% crude protein (CON) when bacteria differed

Correlations Between Intestinal Microbial Species and Jejunum Morphological Traits

Numerous correlations via Spearman's correlation analyses (P<0.05, *Fig. 3*) were investigated between the different genera (n=6) relative abundances and morphological parameters (n=7). *Clostridium*_sensu_stricto_1 was positively correlated with villus width, crypt depth, and muscular thickness, while being negatively correlated with villus height, and ratio of villus height: crypt depth. *Escherichia-Shigella* was positively correlated with muscular thickness and negatively correlated with villus height. *Turicibacter* was positively correlated with crypt depth and muscular thickness, while *Veillonella* was positively correlated with villus width. *Lactobacillus* was positively correlated with villus height, and villus height: crypt depth, and negatively correlated with jejunum weight, villus width, crypt depth, and muscular thickness.

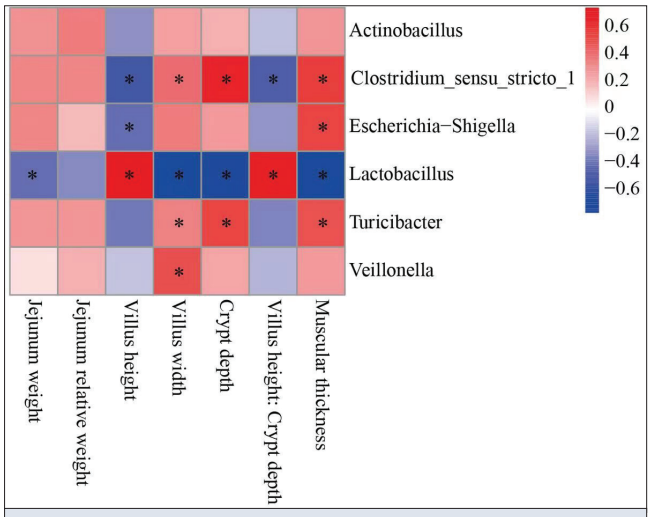

Fig 3. Correlations between differential genera and morphological traits at the jejunum by 21-day old suckling Bamei piglets when feeding maternal diets containing 12% (LP; N=12) or 14% crude protein (CON; N=12). Each row in the graph represents a genus, each column represents a morphological trait, and each lattice represents a Spearman correlation coefficient between a genus and a morphological trait. Red represents a positive correlation, while blue represents a negative correlation. *Significant correlation between the LP and CON groups (P<0.05)

Discussion

The small intestine has an important role in defense against health challenges in addition to nutrient digestion and absorption. The main nutrient digestion and absorption site is the jejunum [23]. Maternal suckled milk enters the piglet's gastrointestinal tract, thereby promoting crypt cell proliferation and proliferation. Suckling piglet jejunal development directly affects post-weaning growth performance [24]. In this study, reducing maternal dietary protein concentrations by 2% units resulted in similar 21 d ADG. The small intestinal growth rate before and after birth of the piglet is greater than the whole body [25]. The small intestine relative weight 24 h after birth is 50% greater than at birth [26]. Intestinal crypt depth increases 40% and villus height increases 35% within 3d [27]. These crypt stem cells divide and differentiate to form intestinal epithelial cells that gradually migrate to the villi tip for nutrient absorption [28]. Through this process, the digestive and absorption functions of intestinal epithelial cells are gradually improved [29].

After the piglet's birth, there are 2 sources of gut microbes with one being the maternal microbes, which are vertically passed, while the 2nd source is environmental, which are horizontally passed. The combined data using Bamei piglets demonstrated that maternal dietary LP concentrations

resulted in significant changes in intestinal microbiome composition compared with CON piglets. Alpha diversity metrics (Shannon and Simpson index) demonstrated a higher piglet bacterial diversity from sows fed lower maternal dietary CP concentrations compared with piglets from sows fed the CON CP concentrations, suggesting that altering CP concentration has a direct impact on jejunal microbial composition of Bamei suckling piglets. In agreement with previous pig studies [30,31], the Bamei piglet's dominant jejunum core microbiome was the phyla Firmicutes, *Proteobacteria*, and *Bacteroidetes*. The dominant genus level Bamei suckling piglet jejunum bacteria were: *Lactobacillus, Escherichia-Shigella*, *Actinobacillus, Buchnera, Romboutsia,* and *Clostridium*_sensu_stricto_1. The bacterial community diversity and richness are known to be influenced by dietary intervention [32].

The correlation analysis between intestinal bacteria *(Clostridium*_sensu_stricto_1, *Lactobacillus,* and *Turicibacter)* and intestinal histomorphology demonstrated that feeding a maternal LP diet can induce shifting abundance changes in the piglet's intestinal microbiome. Equally important, dietary interventions may not always alter the piglet's bacterial species and abundance but may alter the intestinal histomorphology produced by these bacterial species thru influencing their metabolism and physiology. *Lactobacilli* are beneficial bacterial members of the small intestinal microbiota that were reduced for piglets from sows fed the LP diet. The intestinal bacterial environment can protect the intestine from toxic dietary ingredients [33]. The reduction of Lactobacillus spp. abundance may result from decreased oligosaccharide ingestion (less soybean meal inclusion), which reduces nutrient availability, which relates to reduced piglet weight [34]. These results indicate that maternal dietary LP concentration alters Bamei piglets' intestinal microbiota through altering the beneficial bacterial colony structure [35]. Therefore, it is reasonable to hypothesize that intestinal microbiota differences are the result of early dietary intervention, host-microbe interactions, and/or host physiological state. The most important host-microbe interaction may occur on or at the intestinal barrier. These data demonstrated that dietary CP concentrations altered the intestinal microbiome composition and associated function in Bamei piglets. This could be an exciting research field with the potential to solve many important problems.

Availability of Data and Materials

The authors declare that data supporting the study findings are also available to the corresponding authors (J. Jin, J. Jia).

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Ethical Approval

All procedures involving the use of animals were approved by the Animal Care Committee of Qinghai University, China (QHDX-17-02-12-06). Animal slaughtering was approved by the National Administration of Slaughtering and Quarantine regulations (Qinghai, China).

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

Cui YF and Zhang HX: the hypothesis of this study; Cui YF and Zhang LP: work management, article writing; Cui YF, Chen Q and Ren L: experimental procedure follow-up, statistical analysis; Cui YF, Chen Q and Ren L: literature review, review of results; Jia JL: final decision, experimental design.

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