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Changes in Gut Microbiota in a Peruvian Cattle Genetic Nucleus by Breed and Correlations with Beef Quality

Carlos Quilcate [1], Richard Estrada [1,*], Yolanda Romero [1], Diorman Rojas [1], Rolando Mamani [1], Renán Dilton Hañari-Quispe [2], Mery Aliaga [2], Walter Galindo [2], Héctor V. Vásquez [3], Jorge L. Maicelo [3] and Carlos I. Arbizu [3,*]

[1] Dirección de Desarrollo Tecnológico Agrario, Instituto Nacional de Innovación Agraria (INIA), Lima, Peru; ceqp2374@yahoo.com (C.Q.); yolanda.bioinfo@gmail.com (Y.R.); dcasanova@inia.gob.pe (D.C.); diormanr@gmail.com (D.R.); rmamani070@gmail.com (R.M.)

[2] Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional del Altiplano, P.O. Box 291, Puno-Perú.; rhanari@unap.edu.pe (R.D.H-Q.); mlaliaga@unap.edu.pe (M.A.); waltergalindos@gmail.com (W.G.)

[3] Facultad de Ingeniería Zootecnista, Agronegocios y Biotecnología, Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas (UNTRM), Cl. Higos Urco 342, Chachapoyas 01001, Peru; hvasquez@untrm.edu.pe (H.V.V.); jmaicelo@untrm.edu.pe (J.L.M.)

* Correspondence: richard.estrada.bioinfo@gmail.com (R.E.); carbizu@untrm.edu.pe (C.I.A.)

Simple Summary: Understanding the gut microbiota—the community of microorganisms living in the digestive systems of animals—is crucial for improving cattle health and meat quality. This study examined how different breeds of cattle, including Angus, Simmental, Braunvieh, Brahman and crossbreeds Brahman-Simmental F1(SMxBR), have unique gut microbiota compositions that impact their health and meat production. By analyzing the gut bacteria, fungi, and protists, the research identified significant links between the diversity of these microorganisms and important traits such as skin condition, fat distribution, and muscle quality. The study suggests that both breed and environmental factors shape the gut microbiota, influencing the overall health and productivity of cattle. This research is a step towards more efficient and sustainable cattle farming practices, benefiting both the industry and consumers.

Abstract: This study investigates the correlation between gut microbiota diversity and various hematological and meat quality parameters in different cattle breeds, including Angus, Simmental, Braunvieh, Brahman and F1(SMxBR). Using 16S rRNA and 18S rRNA gene sequencing, microbial communities were profiled to discern breed-specific impacts on gut microbiota. Significant correlations were identified between gut microbiota diversity and health parameters, emphasizing the role of microbial diversity in influencing skin condition, fat deposition, and muscle quality. Key bacterial genera such as *Blautia*, *Christensenellaceae_R-7_group*, and *Coprococcus* exhibited significant correlations with meat quality traits. In fungi, genera like *Rhodotorula* exhibited positive correlations with muscle quality, while protist genera such as *Cryptosporidium* negatively correlated with meat quality parameters. These findings suggest that host genetics significantly shape the gut microbiota and highlight the potential for targeted microbiota management to optimize gut health, enhance feed efficiency, and improve meat quality in livestock.

Keywords: gut microbiota; cattle breeds; microbial diversity; meat quality; hematological parameters; host genetics; 16S/18S rRNA

1. Introduction

The normal intestine contains more than 100 trillion microorganisms, mostly bacteria (98%), as well as fungi, viruses, protists, and archaea, establishing a symbiotic relationship with the host that

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is essential for nutrient absorption, immune system regulation, and the maintenance of intestinal barrier integrity [1,2]. The gut microbiota, in addition to producing beneficial substances such as antimicrobial peptides, vitamins, and enzymes, is influenced by the host species and their health status [3]. Recent studies have indicated that host genetics can influence the composition of the rumen microbiota, which is related to rumen metabolites, feed efficiency, and milk quality [4–7].

Intestinal microbial communities play a fundamental role in nutrient digestion, energy harvesting, immune system regulation, and disease development [8]. Variation in the composition and function of the gut microbiota has been associated with the health and productive performance of farm animals [9]. Both environmental factors (such as diet, medication, and hygiene conditions) and host genetic factors (including hereditary genetics, sex, and age) can shape these microbial communities [10]. Evidence has highlighted the diversity of the gut microbiome in different animal breeds fed under identical conditions, identifying breed-specific biomarkers in pigs and broiler chickens [11–13]. Additionally, research has explored rumen microbial differences driven by various breeds and crossbreeding for cattle combinations, indicating significant impacts on microbiota and metabolites [14–17]. These findings underscore the complex interplay between host genetics, gut microbiota, and phenotypic traits, which is crucial for the livestock industry.

The quality of beef is crucial for the livestock industry, as it directly affects consumer satisfaction and market value. Genetic and environmental factors play a significant role in determining essential attributes such as marbling, tenderness, and flavor, which can optimize breeding practices and improve production efficiency [18]. Enhancing beef quality also promotes sustainable management by enabling efficient resource use and improving animal welfare [19]. Characteristics such as backfat thickness, intramuscular fat, and tenderness are related to lipid metabolism and fat deposition [20,21]. Sensory quality, which includes attributes like color, flavor, pH, drip loss, marbling, tenderness, and juiciness, is essential for consumer satisfaction [22]. Additionally, beef quality encompasses processing quality, nutritional value, and hygiene standards [23].

Cattle farming in Peru has advanced significantly due to the introduction of various breeds with valuable attributes for production. In 1993, the Peruvian government created a herd that included the Brahman, Braunvieh, Gyr, and Simmental breeds. The purpose was to promote research in reproductive technologies such as artificial insemination and embryo transfer [24]. Currently, the Estación Experimental Agraria Donoso (EEA Donoso) of INIA in Huaral, under the supervision of the Ministry of Agriculture and Irrigation, is dedicated to the production of in vivo embryos of the Brahman, Gir, Fleckvieh, and Braunvieh breeds with the aim of promoting agricultural development [25]. The Angus breed, recognized worldwide for its high-quality marbled meat, is distinguished by its tenderness and flavor, making it highly valued in the meat industry [26]. The Simmental breed, originating from Switzerland, is appreciated for its robustness and high milk production, also contributing to the quality of its meat [27]. Similarly, the Braunvieh breed, also from Switzerland, is known for its dual purpose, providing both milk and high-quality beef, with a balanced distribution of fat and tenderness [28]. Finally, the Brahman breed, originating from India, is highly resistant to heat and parasites, making it ideal for tropical climates [29]; its crossbreeding with Simmental enhances these traits along with meat quality.

The 16S rRNA gene is a key molecular marker used to explore the ruminal microbiota, offering valuable insights into the structure, taxonomy, and diversity of microbial communities [30]. Similarly, the 18S rRNA marker is vital for studying the intestinal microbiota of fungi and protists due to its conserved nature among eukaryotes, enabling accurate identification and classification [31]. When combined with high-throughput sequencing technologies such as Illumina, this marker facilitates detailed profiling of microbial communities, including those with low abundance [32]. This comprehensive methodology is essential for linking microbial diversity to host health, thereby supporting the development of targeted probiotic therapies to enhance animal health and productivity [33].

This study aims to elucidate the alterations in gut microbiota across various cattle breeds within a genetic nucleus and their associations with beef quality. By analyzing breeds such as Angus, Simmental, Braunvieh, Brahman, and a Simmental-Brahman crossbreed, we intend to discern how

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breed-specific microbial communities' impact critical beef quality attributes. This research will employ molecular markers, specifically the 16S rRNA and 18S rRNA genes, alongside high-throughput sequencing technologies to comprehensively profile microbial communities. The insights derived from this study are anticipated to inform the development of targeted probiotic interventions, thereby enhancing animal health and productivity.

2. Materials and Methods

2.1. Animal Experiment and Sample Collection

In the Huaral region of Lima, situated at an elevation of 128 meters above sea level (coordinates 11°31'18" S and 77°14'06" W), a total of 23 fecal samples were collected from healthy cattle (13 females and 10 males) of 4 different breeds (7 Angus, 5 Braunvieh, 4 Brahman, and 3 Simmental) and 4 crossbreeds F1(SMxBR) from the EEA Donoso. All the cattle were one year and six months old and had lived under the same conditions.

The diet at the EEA Donoso mainly consists of fresh forages, supplemented with specific additives (Table 1). Fecal samples were collected from the rectum of each animal using disposable gloves. These samples were promptly transported to the laboratory in liquid nitrogen and stored at -80°C until DNA extraction. The veterinary team at the EEA Donoso Genetic Center in Huaral regularly monitored the animals, conducting parasitological examinations that confirmed the absence of cysts, oocysts, or larvae. Routine veterinary evaluations, including physical exams, medical history assessments, and laboratory tests, were performed to ensure high health standards. As a result, there were no sick animals in the genetic nucleus. This study was carried out in accordance with Peruvian National Law No. 30407: "Animal Protection and Welfare."

Table 1. Diet of Cattle at EEA Donoso.

Feed Type	Amount	Dry Matter	Moisture
Corn Silage	3% of body weight	24%-26%	74%-76%
Balanced Feed	2 kg	90%	10%
Crude Protein	14%		

2.2. Meat Quality Traits Detection

To acquire the ultrasound images, the cattle were immobilized and secured by the head in a squeeze chute. The imaging sites were identified through physical palpation to ensure precise determination of the scanning locations. The animals were manually restrained, ensuring that no abnormal conditions arose that could cause stress. Ultrasound scanning was performed only when the animals were in a relaxed posture, allowing for accurate measurements. Ultrasound imaging was performed to assess the Loin skin thickness (GPL), loin fat thickness (GGL), loin thickness (GL), hip skin thickness (GPC), hip fat thickness (GGC), marbled beef ultrasonography right buttock (NMG1), marbled beef ultrasonography right buttock (NMG2), and Loin area (AL), were measure in vivo using an ESAOTE (Esaote Pie Medical, Aquila Vet model, with a 6 MHz linear transducer, Maastrich, The Netherlands) ultrasound machine equipped with an APS 3.5 MHz transducer. To enhance image clarity, the measurement site was shaved, cleaned, and lubricated with vegetable oil, and a soft material guide was employed to improve contact between the transducer and the animal's curved body surfaces. The weight of the animal was measured at the same time of the ultrasonography.

2.3. Analyses of Blood Parameters

Blood samples were collected from all cattle via jugular venipuncture. A total of 23 samples were analyzed for red blood cell count, white blood cell count, and platelets using the Dx® hematology analyzer (IDEXX Laboratories, Westbrook, MA, USA). Additionally, 23 plasma samples were analyzed for triglyceride levels using the Beckman-CX4 automatic biochemical analyzer (Beckman Coulter, Inc., Brea, CA, USA). Detailed information on the variables analyzed is provided in Supplementary Table S1

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2.4. DNA Extraction and Sequencing

The genomic DNA from each fecal sample was extracted utilizing the QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA, USA). The DNA concentration and purity were assessed with a NanoDrop 2000 spectrophotometer, and the 260/280 absorbance ratio was measured at the same time (Thermo Fisher Scientific, USA). Additionally, the integrity of the DNA was confirmed by 0.8% agarose gel electrophoresis. To construct the Illumina amplicon sequencing library, approximately 10 ng of DNA from each sample were used for PCR amplification with primers 515F/806R for 16S rRNA and 528F/706R for 18S rRNA. The PCR conditions for 16S rRNA amplification protocol consisted of an initial denaturation at 94 °C for 1 minute, followed by 30 cycles of denaturation at 94 °C for 20 seconds, annealing at 54 °C for 30 seconds, and elongation at 72 °C for 30 seconds, with a final extension at 72 °C for 5 minutes. For 18S rRNA, the protocol began with an initial denaturation at 94°C for 2 minutes, followed by an initial set of 5 cycles consisting of denaturation at 94°C for 45 seconds, annealing at 52/54°C for 45 seconds each, and elongation at 72°C for 1 minute. This was followed by 35 additional cycles with a reduced annealing temperature of 50/52°C, and concluded with a final elongation step at 72°C for 10 minutes. Sequencing libraries were prepared using the Illumina TruSeq DNA PCR-Free Library Preparation Kit (Illumina, USA) as per the protocol, which included the addition of index sequences. The quality of the libraries was assessed with a Qubit 2.0 fluorometer (Thermo Scientific). The validated libraries were then sequenced by the sequencing service of Novogene (USA) on the Illumina NovaSeq 6000 platform with 250 bp paired-end reads (Illumina Inc., San Diego, CA, USA) following the manufacturer's instructions.

2.5. Bioinformatics Analysis

In the QIIME2 analysis, the data underwent trimming and quality filtering. Paired-end reads, demultiplexed by Illumina, were then used to create an Amplicon Sequence Variants (ASVs) table using the qiime2-dada2 plugin. To reduce the number of false positive ASVs, sequences with fewer than 10 reads in total across all samples were removed. Additionally, sequences of plant origin were excluded. Taxonomic classification of the ASVs was conducted using the SILVA v138.1 database, which provided the basis for identifying bacteria through 16S sequences and fungi and protists through 18S sequences. The high-quality filtered sequences were subsequently aligned using the integrated MAFFT aligner. Using the QIIME2 phylogenetic module, both rooted and unrooted phylogenetic trees were constructed for bacteria, fungi, and protists with the FastTree algorithm.

2.6. Statistics Analysis

The dataset was analyzed statistically using R software v.4.1.1 [34] with the Phyloseq [35], Microeco [36] and MicrobiotaProcess [37] packages. Initially, rarefaction curves were generated for each sample to assess sequencing depth. Various alpha diversity indices, including Observed, Chao1, ACE, Pielou, Simpson, and Shannon, were calculated to evaluate the diversity of intestinal bacteria, fungi, and protists. Beta diversity was assessed using the Jaccard and Unweighted Unifrac methods, and the results were visualized through Principal Coordinate Analysis (PCoA). To examine differences in microbial communities among groups, a two-way PERMANOVA test with 9999 permutations was conducted. A linear discriminant analysis (LDA) with LEfSe, an algorithm for identifying biomarkers of significant statistical and biological importance, was conducted. Additionally, Spearman rank correlation analyses were performed to explore relationships between blood and meat quality parameters and alpha diversity indices. Finally, Mantel tests with 999 permutations were used to investigate the association between variables and community composition.

3. Results

In the bacteria, 32,670,654 high-quality filtered reads were obtained. On average, 142,046 high-quality reads were recorded per sample, reaching a maximum of 2,159,093 and a minimum of 775,522 high-quality reads. In fungi, 6,720,444 high-quality filtered reads were obtained. On average, 29,219

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high-quality reads were recorded per sample, reaching a maximum of 849,583 and a minimum of 77,722 high-quality reads. In protists, 20,393,204 high-quality filtered reads were obtained. On average, 88,666 high-quality reads were recorded per sample, reaching a maximum of 1,466,373 and a minimum of 151,082 high-quality reads. The rarefaction curves (Fig. S1) indicated that the sampling depth was adequate to capture the biodiversity within the dataset.

3.1. Analysis of Effect of Breed on the Gut Microbiota Diversity and Composition

The ACE, Chao1, Observed, Pielou, Shannon, and Simpson diversity indices were calculated for bacteria, fungi (Figure 1), and protists (Fig. S2) in the communities of different cattle breeds, revealing clear differences. In bacterial communities (Figure 1A), significant differences were identified between Angus and Brahman for ACE (p = 0.042), Shannon (p = 0.042), and Simpson (p = 0.042). Significant differences were detected between Angus and Braunvieh in ACE (p = 0.0025), Chao1 (p = 0.0025), Observed (p = 0.0057), Pielou (p = 0.0025), Shannon (p = 0.0025), and Simpson (p = 0.0025). Angus and F1(SMxBR) exhibited significant differences in Pielou (p = 0.012), Shannon (p = 0.024), and Simpson (p = 0.042). Significant differences were recorded between Angus and Simmental in ACE (p = 0.017), Chao1 (p = 0.017), Observed (p = 0.022), Pielou (p = 0.033), Shannon (p = 0.017), and Simpson (p = 0.033). Differences in ACE (p = 0.032), Chao1 (p = 0.032), Observed (p = 0.032), and Shannon (p = 0.032) were significant between Brahman and Braunvieh.

Regarding fungi (Figure 1B), significant differences were identified between Angus and Brahman in ACE (p = 0.012), Chao1 (p = 0.0061), and Observed (p = 0.0061). Significant differences were detected between Angus and Braunvieh in ACE (p = 0.0025), Chao1 (p = 0.0057), and Observed (p = 0.0056). Significant differences were recorded between Brahman and Braunvieh in ACE (p = 0.029), Chao1 (p = 0.029), and Observed (p = 0.029). Differences in ACE (p = 0.036), Chao1 (p = 0.036), and Observed (p = 0.035) were significant between Braunvieh and Simmental. Finally, significant differences were identified between Braunvieh and F1(SMxBR) in ACE (p = 0.016), Chao1 (p = 0.019), and Observed (p = 0.019).

In protists (Fig. S2), significant differences were identified between Angus and Brahman in Chao1 (p = 0.042), Observed (p = 0.012), Pielou (p = 0.0061), Shannon (p = 0.0061), and Simpson (p = 0.0061). Significant differences were detected between Angus and Braunvieh in Pielou (p = 0.01), Shannon (p = 0.0025), and Simpson (p = 0.01). Significant differences were recorded between Brahman and F1(SMxBR) in Observed (p = 0.029), Pielou (p = 0.029), Shannon (p = 0.029), and Simpson (p = 0.029). Finally, significant differences were identified between Braunvieh and F1(SMxBR) in Pielou (p = 0.016), Shannon (p = 0.016), and Simpson (p = 0.016).

In beta diversity analysis of gut microbiota communities in cattle, using unweighted UniFrac and Jaccard metrics, a PCoA plot demonstrated that the composition of microbial communities was influenced by breed and sex (Figure 2). For both UniFrac and Jaccard unweighted metrics in bacteria have been influenced by race and sex (Figure 2A,B), but only in Jaccard, community composition was significantly affected by the interaction of race and sex (p = 0.045), as verified by PERMANOVA (Table 2). In fungal composition, the Unweighted UniFrac metric (Figure 2C) revealed a significant effect of sex (p = 0.027), while the Jaccard metric (Figure 2D) indicated significant effects of both breed (p = 0.001) and sex (p = 0.036). Lastly, in the protist composition, the Unweighted UniFrac (p = 0.004) and Jaccard (p = 0.003) PCoA (Figure 2E,F) plots indicated that the community was significantly influenced by breed.

Table 2. PERMANOVA of Unweighted Unifrac and Jaccard methods.

	Bacteria				**Fungi**				**Protists**			
	Unweighted unifrac		Jaccard		Unweighted unifrac		Jaccard		Unweighted unifrac		Jaccard	
Items	R^2	p	R^2	p	R^2	p	R^2	p	R^2	p	R^2	p
Race	0.22	0.001**	0.22	0.001**	0.21	0.058	0.22	0.001**	0.27	0.004**	0.23	0.003**

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Sex	0.05	0.022*	0.05	0.035*	0.07	0.027*	0.05	0.036*	0.05	0.225	0.05	0.058
Race:Sex	0.19	0.083	0.19	0.045*	0.15	0.691	0.17	0.438	0.2	0.1	0.17	0.082

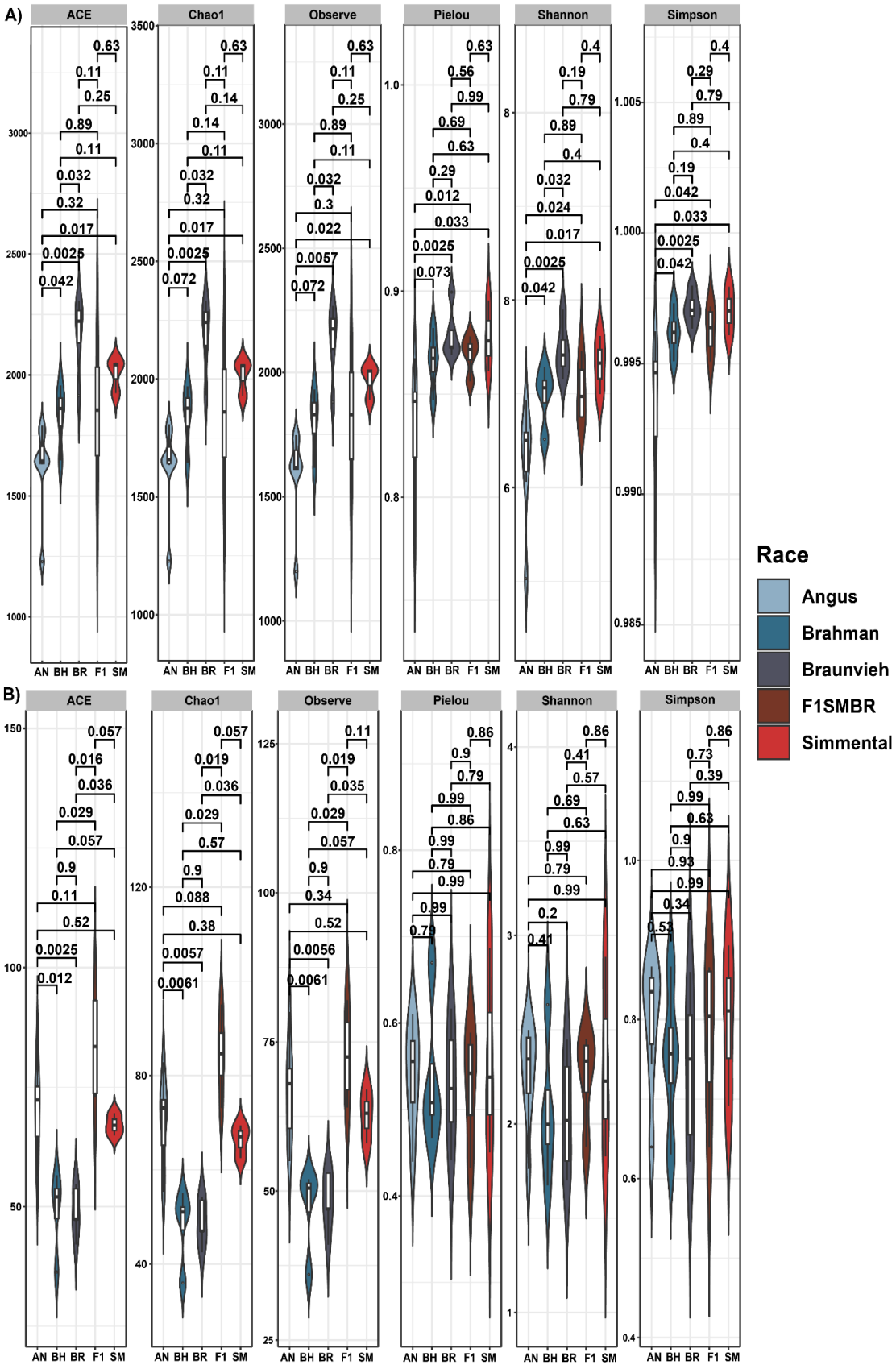

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Figure 2. Alpha diversity metrics of bacteria and fungi were evaluated in cattle in five breeds. Diversity indices exhibited include ACE, Chao1, Observe, Pielou, Shannon and Simpson. A) Alpha diversity of bacteria B) Alpha diversity of fungi.

Venn diagrams illustrate ASVs of bacteria, fungi and protists, both unique and shared, among cattle of different breeds (Fig. S3). For bacterial ASVs (Fig. S3A), 1483 are common to all breeds, while 1569 are unique to Braunvieh, 1092 to Brahman, 1686 to Angus, 722 to Simmental and 784 to F1(SMxBR). In the fungal ASVs (Fig. S3B), 43 are common to all breeds, with 36 unique to Braunvieh, 30 to Brahman, 97 to Angus, 39 to Simmental, and 49 to F1(SMxBR). For protist ASVs (Fig. S3C), 35 are common to all breeds, while 93 are unique to Braunvieh, 40 to Brahman, 109 to Angus, 42 to Simmental, and 61 to F1(SMxBR).

3.2. Effect of Breed on the Gut Microbiota Taxonomy

At the phylum level, Firmicutes were the dominant bacterial phylum (Figure 4A), comprising 69% in Angus, 67% in Brahman, 67% in Braunvieh, 65% in Simmental, and 65% in F1(SMxBR). Bacteroidota accounted for 26%, 32%, 31%, 33%, and 31% in Angus, Brahman, Braunvieh, Simmental, and F1(SMxBR), respectively. All other bacterial taxa collectively represented 5%. The dominant fungal phylum (Figure 4C) was Ascomycota, with representations of 95% in Angus, 97% in Brahman, 93% in Braunvieh, 95% in Simmental, and 88% in F1(SMxBR). All other fungal phyla collectively represented 5%. Among protists (Fig. S4A), the dominant phyla were Ciliophora and Incertae Sedis. Ciliophora constituted 46% in Angus, 67% in Brahman, 85% in Braunvieh, 4% in Simmental, and 22% in F1(SMxBR). Incertae Sedis were represented by 36% in Angus, 27% in Brahman, 12% in Braunvieh, 84% in Simmental, and 55% in F1(SMxBR). Chlorophyta represented 7%, 3%, 2%, 7%, and 12% in Angus, Brahman, Braunvieh, Simmental, and F1(SMxBR), respectively. Apicomplexa accounted for 9%, 2%, 1%, 3%, and 7% in Angus, Brahman, Braunvieh, Simmental, and F1(SMxBR), respectively. All other protist phyla collectively represented 5%.

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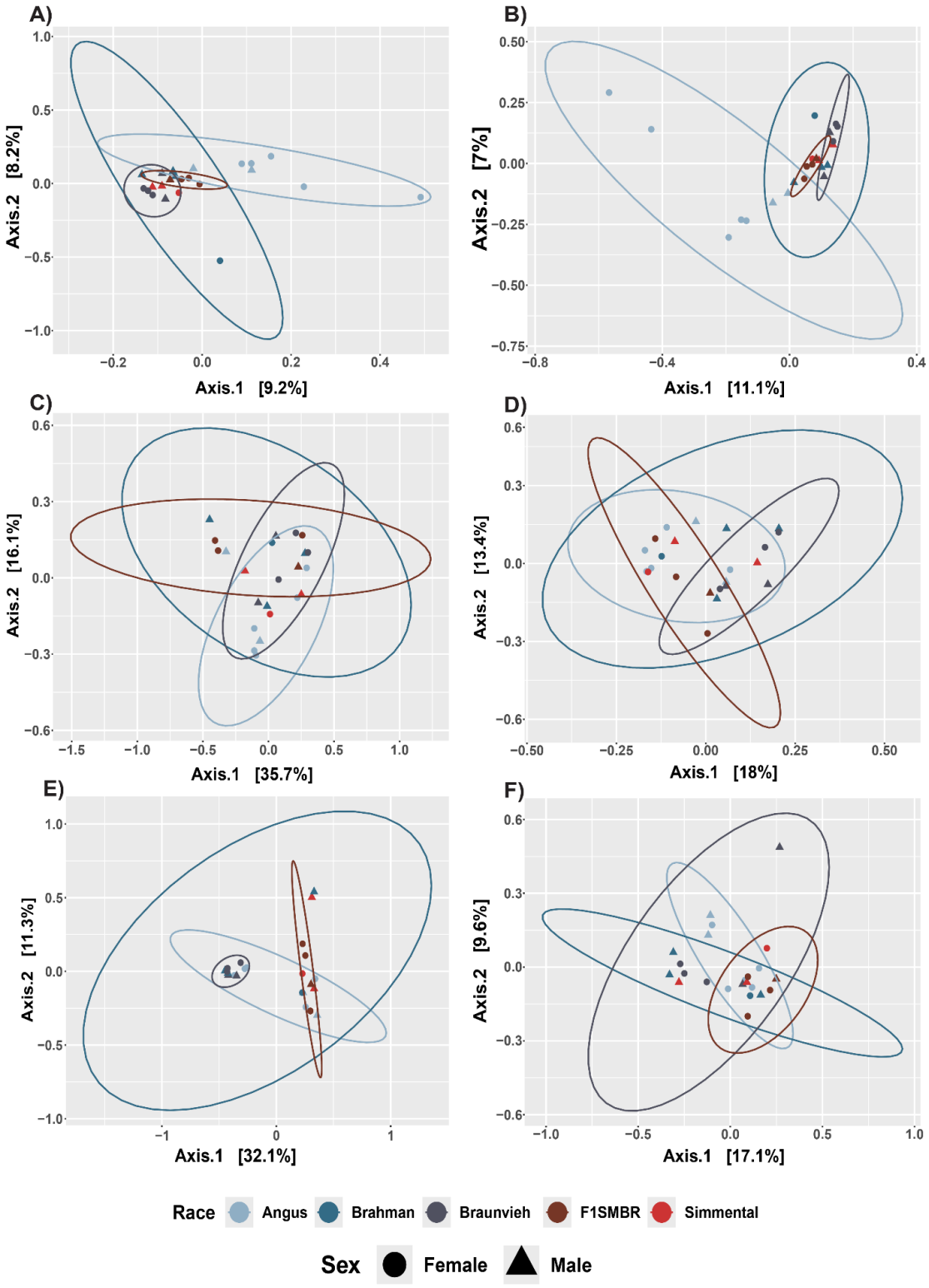

Figure 3. Unweighted UniFrac and Jaccard analysis of beta diversity in the different breeds of cattle. A) Unweighted Unifrac of bacteria B) Jaccard of bacteria C) Unweighted Unifrac of fungi. D) Fungi Jaccard E) Protist Unweighted Unifrac F) Protist Jaccard.

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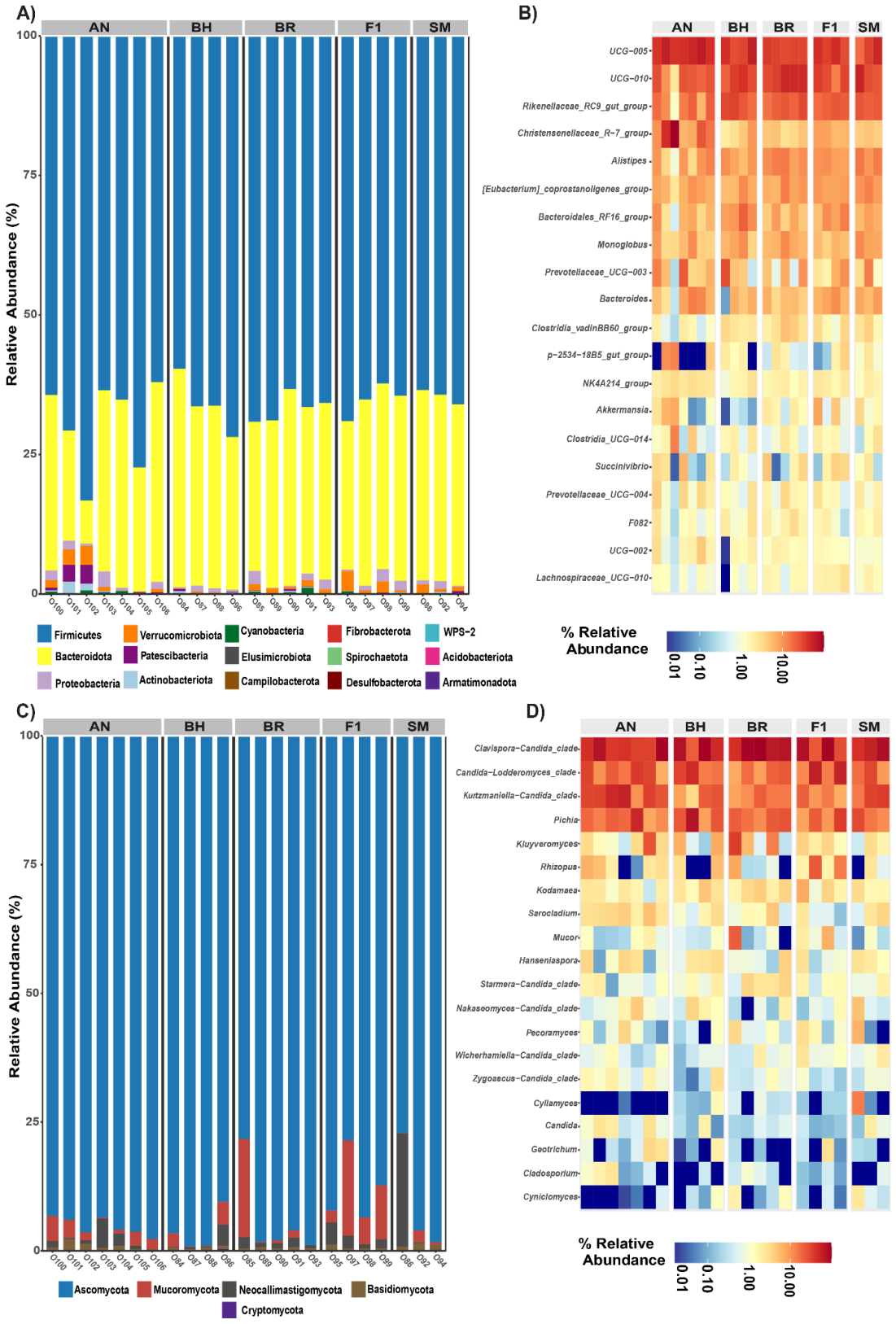

Figure 4. Relative abundances in the gut microbiota at the phylum and genus level in different cattle breeds. A) Bar graph analysis illustrates the abundance of bacterial phyla in each breed. B) Heat map with the main bacterial abundances of the 20 genera in each breed. C) Bar graph analysis illustrates the abundance of fungal phyla in each breed; D) Heat map with the main abundances of fungi of 20 genera in each breed.

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The heatmap illustrates the relative abundance of various genera across different breeds (Figure 4B,D). The 20 most abundant genera were observed. In the analysis of bacterial composition at the genus level (Figure 4B), the most abundant genera observed were *UCG-005*, *UCG-010*, *Rikenellaceae_RC9_gut_group*, *Christensenellaceae_R-7_group*, and *Alistipes*. Regarding fungal composition (Figure 4D), the predominant genera were *Clavispora-Candida_clade*, *Candida-Lodderomyces_clade*, *Kurtzmaniella-Candida_clade*, and *Pichia*. In the protist composition (Fig. S4B), the most abundant genera were *Trichostomatia*, *Blastocystis*, *Trebouxiophyceae*, and *Gregarina*.

3.3. Relationship between Gut Microbiota and Beef Quality Variables

The Spearman correlation analysis between meat quality variables and the relative abundances of intestinal microbiota genera revealed several significant associations (Figure 5). Among the bacterial genera (Figure 5A), NGM1 and NGM2 exhibited significant positive correlations with NK4A214_group and *Christensenellaceae_R-7_group*, and significant negative correlations with *Odoribacter*, *Clostridia_vadinBB60_group*, *Coprococcus*, *gir-aah93h0*, and *Agathobacter*. Additionally, NGM2 was significantly negatively correlated with *[Clostridium]_methylpentosum_group*. GPL demonstrated a significant negative correlation with *Bifidobacterium* and a positive correlation with *Hydrogenoanaerobacterium*. GGL and GGC were negatively correlated with *Negativibacillus* and *Lachnospiraceae_UCG-010*, with GGC also exhibiting a significant negative correlation with *Veillonellaceae_UCG-001*. GPC was negatively correlated with *Blautia*, *Christensenellaceae_R-7_group*, and *Veillonellaceae_UCG-001*. Body weight exhibited significant negative correlations with *Lachnospiraceae_UCG-010* and *Veillonellaceae_UCG-001*. GL was negatively correlated with *Lachnospiraceae_UCG-010*. Finally, AL demonstrated significant negative correlations with the *Christensenellaceae_R-7_group* and *Lachnospiraceae_UCG-010*.

Regarding fungal genera (Figure 5B), only *Rhodotorula* exhibited a significant positive correlation with NGM1 and NGM2 and a significant negative correlation with GPL. Among protist genera (Figure 5C), NGM1 and NGM2 had significant negative correlations with *Pseudoperkinsidae* and *Colpodida*. GL was significantly negatively correlated with *Cryptosporidium*, *Colpodella*, *Tetramitia*, *Trebouxiophyceae*, *Gregarina*, *Colpodida*, *Ochromonas*, *Blastocystis*, and *Cercomonas*, while demonstrating a significant positive correlation with *Trichostomatia*. AL exhibited significant negative correlations with *Cryptosporidium* and *Colpodella*. Lastly, GPC had significant negative correlations with *Cryptosporidium*, *Colpodella*, *Tetramitia*, *Blastocystis*, and *Cercomonas*.

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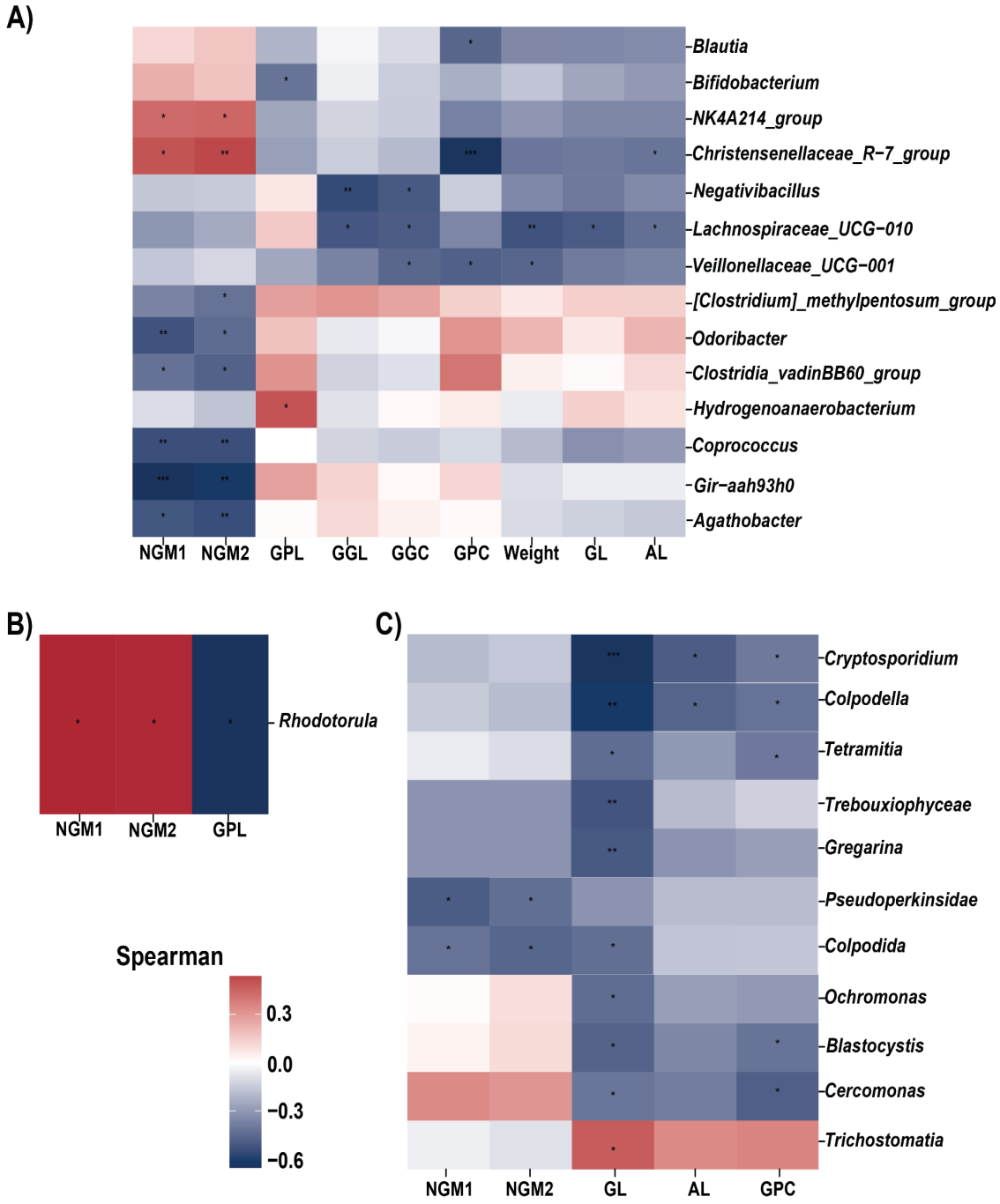

Figure 5. Heat map of Spearman correlation analysis between the genera of intestinal microbes and meat variables. *$p < 0.05$; **$p<0.01$; ***$p<0.001$. A) Heat map of bacterial genera. B) Heat map of fungi genera. C) Heat map of protist genera.

3.4. Biomarkers Identification for Different Breed

To identify specific bacterial taxa associated with different breeds, a comparative analysis of gut microbiota compositions was performed. This analysis was performed using the linear discriminant analysis effect size method (LEfSe). The most significant differentiation in taxa, from phylum to genus level, was determined through an LDA score (Figure 6).

In Angus cattle, the following bacterial taxa were identified (Figure 6A) one phylum (Patescibacteria), two orders (Christensenellales and Saccharimonadales), one class (Saccharimonadia), two families (Christensenellaceae and Saccharimonadaceae), and two genera (*Christensenellaceae_R-7*_group and *Candidatus_Saccharimonas*). In Braunvieh, one genus (*Dorea*) was

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identified. In F1(SMxBR), one order (Lachnospirales), one family (Lachnospiraceae), and two genera (*Lachnospiraceae_UCG-010* and *Coprococcus*) were identified. In Simmental, one family ([Eubacterium]_coprostanoligenes_group) and one genus (*[Eubacterium]_coprostanoligenes*_group) were identified. Regarding fungal biomarkers (Figure 6B), in Angus, one genus (*Candida*) and one species (*Candida cylindracea*) were identified. In Simmental, one genus (*Kurtzmaniella-Candida_clade*) was identified. In Braunvieh, one genus (*Cyllamyces*) was identified.

For protist biomarkers (Figure 6C), in Brahman, one phylum (Ciliophora), one order (Litostomatea), one class (Intramacronucleata), one family (Trichostomatia), one genus (*Trichostomatia*), and one species (*Buxtonella sulcata*) were identified. In F1(SMxBR), two phyla (Apicomplexa and Chlorophyta), one order (Trebouxiophyceae), two classes (Conoidasida and Trebouxiophyceae), one family (Trebouxiophyceae), one genus (*Trebouxiophyceae*), and one species (*Prototheca zopfii*) were identified. In Simmental, one genus (*Blastocystis*) and one species (*Blastocystis* sp.) were identified.

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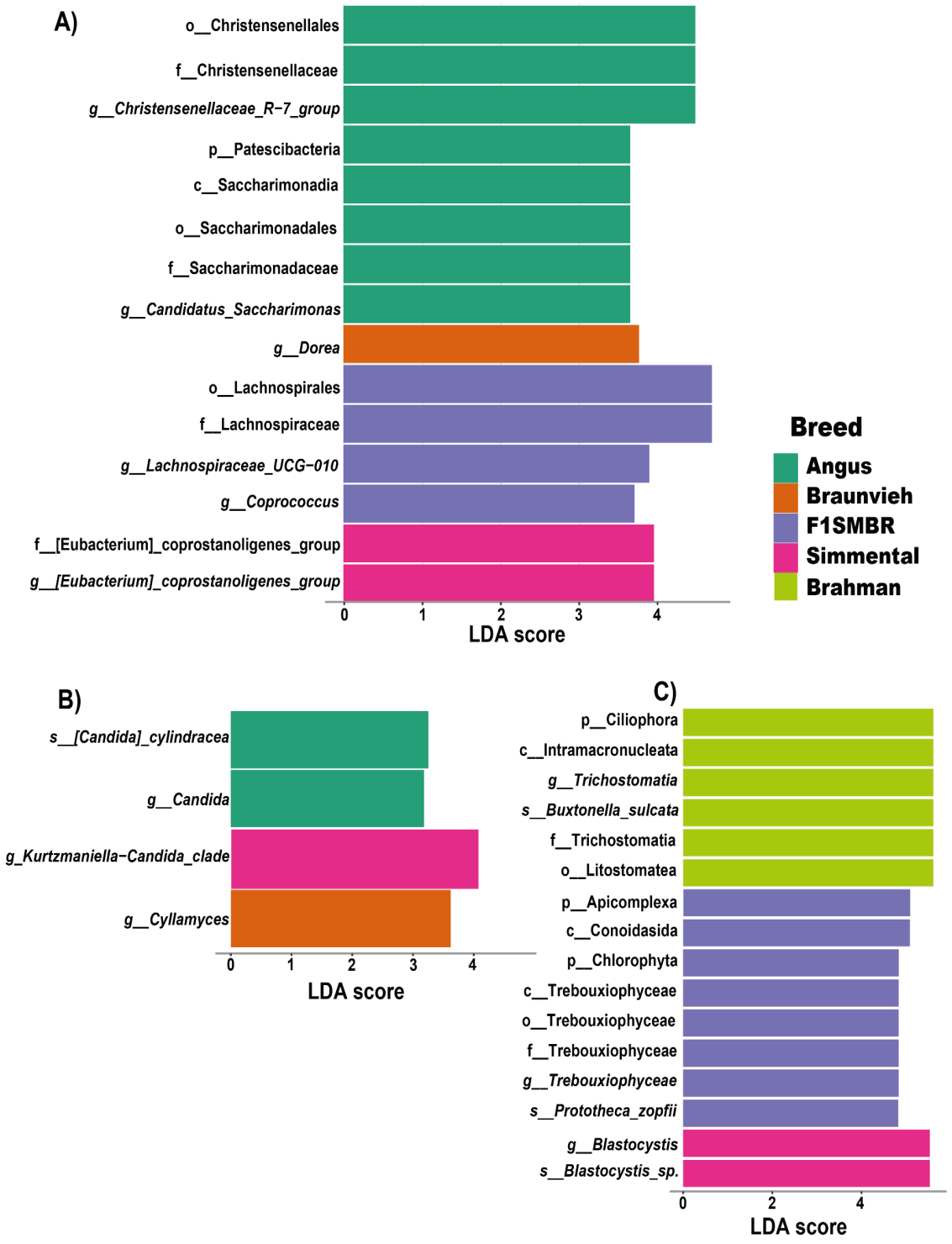

Figure 6. LEfSe analysis of the intestinal microbiome in different breeds. A) LEfSe of bacteria. B) LEfSe of fungi. C) LEfSe of protist.

3.5. Breed Relationship of Alpha/Beta Diversity with Variables

The variables, hematological and quality meat, were analyzed using the Kruskal-Wallis test, with breed as the variable of interest (Table S1). Only significant parameters for breeds included GL, GPC, GGC, NGM1, and NGM2 (Table S2). Subsequently, Bonferroni post-hoc analysis was conducted. For GL, the comparison between Braunvieh and F1(SMxBR) yielded a significance of 0.0175. For GPC, the comparison between Brahman and F1(SMxBR) indicated a significance of 0.0457.

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In the case of GGC, the comparison between Brahman and Braunvieh had a significance of 0.0445, and Brahman versus F1(SMxBR) had a significance of 0.0061. For NGM1, the comparison between Angus and F1(SMxBR) was significant at 0.005. Finally, for NGM2, the comparison between Angus and F1(SMxBR) was significant at 0.0084 (Table S3).

A Spearman correlation analysis was conducted to examine the relationship between variables and alpha diversity indices for bacteria, fungi, and protists (Figure 7). For bacteria (Figure 7A), the Shannon index exhibited a significant positive correlation with GPL. The Simpson index was significantly positively correlated with GPC and significantly negatively correlated with NGM2. The Pielou index demonstrated significant negative correlations with NGM1 and NGM2.

Regarding fungi (Figure 7B), the Observed index had significant positive correlations with MON% and significant negative correlations with EOS, GL, and GPC. The Chao index was significantly positively correlated with MON%, MON, and PLT, and significantly negatively correlated with EOS, GL, AL, and GPC. The ACE index exhibited significant positive correlations with MON% and significant negative correlations with EOS, GL, AL, and GPC. The Shannon and Pielou indices were significantly positively correlated with NGM2. Finally, the Simpson index exhibited significant positive correlations with NGM1 and NGM2.

For protists (Figure 7C), the Observed, Chao1, ACE, Shannon, Simpson, and Pielou indices were significantly positively correlated with PLT. These indices were also significantly negatively correlated with WG, GL, AL, and GPC. Shannon, Simpson, and Pielou indices were significantly positively correlated with MON% and significantly negatively correlated with GGL and GGC. The Observed index was significantly negatively correlated with HGB and GGC. The Chao1 index was significantly negatively correlated with HCT and GPL. Finally, the Simpson index was significantly negatively correlated with EOS.

In the correlation analysis of variables with beta diversity for fungi and protists, several significant results were identified. No significant correlations were observed for bacteria (Table 3). In fungi, the Jaccard index in the Mantel test exhibited significant correlations for NGM1 ($p = 0.016$) and NGM2 ($p = 0.033$). The Partial Mantel test also revealed significant correlations for NGM1 ($p = 0.017$) and NGM2 ($p = 0.036$). For the Unweighted Unifrac index in the Mantel test, significant correlations were identified for WBC ($p = 0.019$), Weight ($p = 0.015$), RBC ($p = 0.031$), MCV ($p = 0.019$), MCHC ($p = 0.025$), NEU ($p = 0.038$), and SEG ($p = 0.039$). In the Partial Mantel test, WBC ($p = 0.041$) and Weight ($p = 0.044$) were also significantly correlated.

Regarding protists, the Jaccard index in the Mantel test exhibited significant correlations for PLT ($p = 0.021$) and NEU% ($p = 0.031$). The Partial Mantel test also identified a significant correlation for PLT ($p = 0.024$). For the Unweighted Unifrac index in the Mantel test, significant correlations were identified for MON ($p = 0.013$), PLT ($p = 0.026$), GL ($p = 0.01$), and AL ($p = 0.033$). The Partial Mantel test revealed significant correlations for MON ($p = 0.008$), PLT ($p = 0.026$), GL ($p = 0.006$), and AL ($p = 0.044$).

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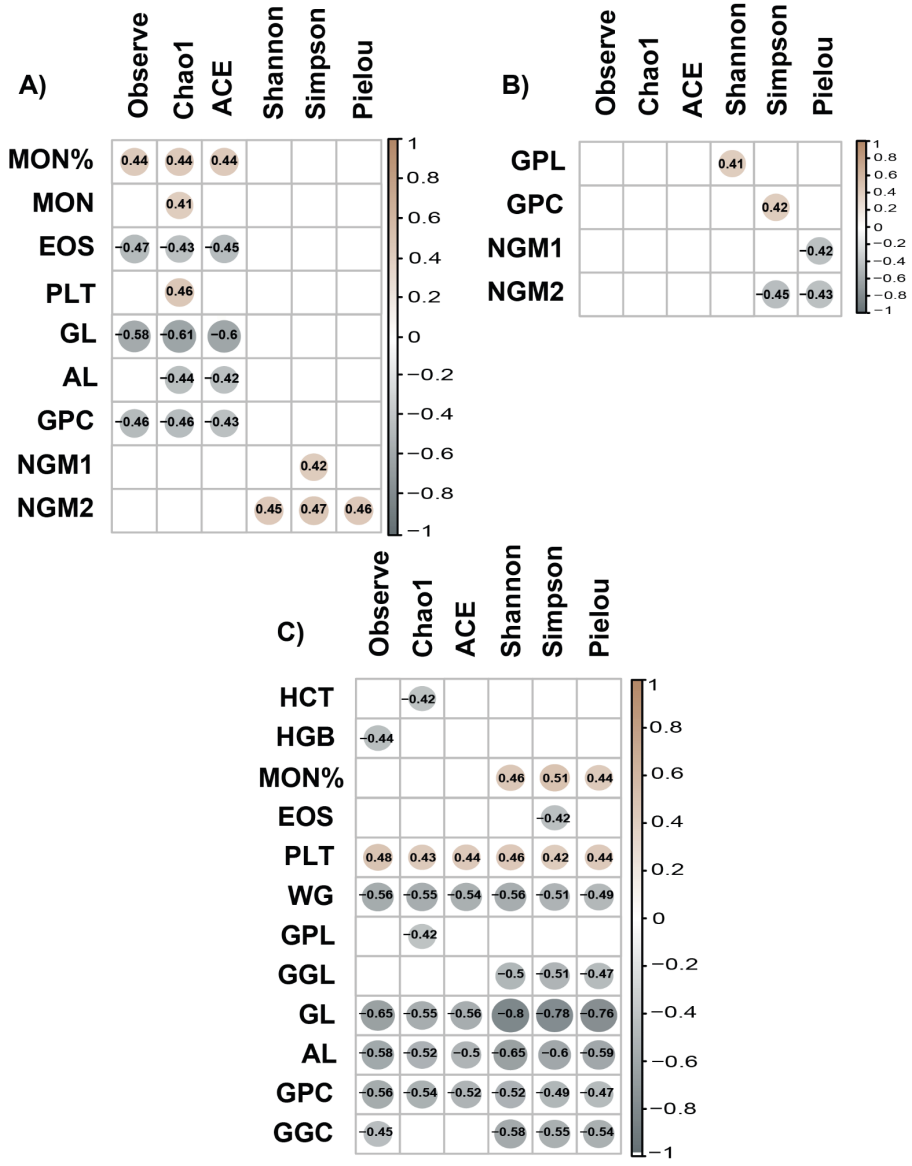

Figure 7. Correlations between the alpha diversity of the intestinal microbiota and hematological and meat variables. A) Spearman correlation of alpha diversity of bacteria. B) Spearman correlation of fungal alpha diversity. C) Spearman correlation of protist alpha diversity.

Table 3. Correlation of variables with Beta diversity (Jaccard and Unweighted Unifrac) using Mantel and Partial Mantel Tests. Only Significant Variables Presented.

Fungi	Jaccard				Unweighted Unifrac			
	Mantel test		Partial Mantel test		Mantel test		Partial Mantel test	
Variables	r	*p*	r	*p*	r	*p*	r	*p*
NGM1	0.22821	0.016	0.23833	*0.017*	-	-	-	-
NGM2	0.19406	0.033	0.20089	*0.036*	-	-	-	-
WBC	-	-	-	-	0.22348	*0.019*	0.1771	*0.041*
WEIGHT	-	-	-	-	0.28379	*0.015*	0.22588	*0.044*
RBC	-	-	-	-	0.26543	*0.031*	-	-
MCV	-	-	-	-	0.27068	*0.019*	-	-
MCHC	-	-	-	-	0.25847	*0.025*	-	-
NEU	-	-	-	-	0.19148	*0.038*	-	-
SEG	-	-	-	-	0.19148	*0.039*	-	-

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Protists	r	*p*	r	*p*	r	*p*	r	*p*
MON	-	-	-	-	0.19531	*0.013*	0.20835	*0.008*
PLT	0.22961	*0.021*	0.2268	*0.024*	0.16086	*0.026*	0.1609	*0.026*
GL	-	-	-	-	0.25734	*0.01*	0.27912	*0.006*
AL	-	-	-	-	0.13362	*0.033*	0.1335	*0.044*
NEU%	0.21743	*0.031*	-	-	-	-	-	-

4. Discussion

In this study, alpha diversity of the intestinal microbiota exhibited significant correlations with various hematological and meat quality parameters across different cattle breeds. Angus cattle exhibited higher alpha diversity indices compared to Brahman, Braunvieh, Simmental, and F1(SMxBR) crosses, with significant differences observed in the ACE, Chao1, Observed, Pielou, Shannon, and Simpson indices. Additionally, significant differences were identified in the taxonomic composition of bacteria, fungi, and protists among the various breeds. These findings underscore the importance of considering genetic and environmental factors, including breed, in shaping the gut microbiota. Therefore, this study was conducted to further understand these interactions and develop strategies to optimize gut health, enhance feed efficiency, and improve meat quality in livestock production.

Clinical studies were carried out that indicated normal hematological values in cattle, indicating that the animals were healthy [38]. Additionally, meat quality analyses demonstrated that parameters such as marbling and fat content were within acceptable ranges [39].

The race exhibited a significant correlation with alpha diversity of the intestinal microbiota, consistent with previous findings in bacteria [3,40], fungi [2,41], and protists [42–44]. These studies underscore the crucial role of genetic and environmental factors in shaping the microbial communities within the gut. The observed differences in alpha diversity among various races suggest that host genetics significantly influence the richness and evenness of gut microbiota. This aligns with evidence from other animal studies, where specific breeds demonstrate distinct microbial profiles due to inherent genetic traits and adaptive responses to their environments [2,3]. In pigs, alpha diversity of gut eukaryotic communities, including fungi and protists, exhibited low but notable heritability, indicating a limited genetic influence on the diversity of these communities [44]. Similarly, in cattle, different breeds exhibit significant variations in rumen bacterial diversity, which can affect nutrient digestion and energy harvest [45,46].

In this study, beta diversity analysis revealed significant variations in the gut microbiota among different breeds. Both breed and the interaction of breed and gender influenced the microbiota composition. These findings are consistent with previous research, highlighting the importance of considering breed and gender in microbiota studies [47,48]. Changes related to breed in the microbiota of cattle are well documented, with specific breeds demonstrating distinct microbial communities [3,46]. For instance, studies have indicated that bacterial diversity in the rumen varies between breeds, with some breeds exhibiting higher diversity associated with lower nutrient utilization efficiency [7]. Similarly, the influence of breed and the interplay between breed and sex has been observed in dogs [49] and pigs [50]. Similarly, the influence of breed on protist and fungal communities was observed, akin to findings in bulls [43], pigs [44], and humans [42]. Additionally, the effect of sex on fungal communities was noted in goats [41] and dogs [51].

These studies underscore the crucial role of breed and sex in shaping gut microbiota, as they highlight significant variations in microbiota composition linked to these biological factors, suggesting their profound impact on the configuration of microbial communities across different species. Understanding these correlations is essential for developing targeted strategies to manipulate gut microbiota for improving health and disease management in different breeds.

All the analyzed breeds exhibited the same predominant bacterial phyla, Firmicutes and Bacteroidota, which is consistent with previous studies in cattle and other ruminants, such as goats [4,52], alpacas [53,54], and cattle [3,55]. Firmicutes, known for their role in fermenting complex carbohydrates and producing short-chain fatty acids, are crucial for the digestive efficiency of

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ruminants [56]. Bacteroidota, on the other hand, are essential for breaking down plant polysaccharides and producing volatile fatty acids [57]. Similarly, all breeds exhibited Ascomycota as the predominant fungal phylum, followed by Mucoromycota, corroborating previous studies in cattle [47,58], alpacas [53], and goats [41]. Ascomycota includes many fungi that decompose organic matter and contribute to the nutrient cycle in the rumen ecosystem [3]. Mucoromycota also plays a role in organic material decomposition and is important in the structure and function of the ruminal microbiome [3]. Additionally, the analysis of protists revealed that the dominant phyla were Ciliophora and Incertae Sedis, which have also been reported in primates [59] and humans [42,60]. Ciliophora, characterized by the presence of cilia, are important in predating bacteria and maintaining microbial balance [61].

The increase in butyrate-producing bacteria, particularly *UCG-010* and *UCG-005*, across different breeds highlights their role in enhancing butyrate production, crucial for intestinal health. These bacteria improve digestion and intestinal maturation by converting lactate into beneficial compounds, thus providing energy and reducing inflammation [62,63]. The presence of *Rikenellaceae RC9* in breeds underscores its importance in breaking down fibrous plant materials, facilitating the degradation of polysaccharides such as starch, cellulose, and lignin in the hindgut [64–66]. Additionally, the genus *Alistipes* was abundantly present across all breeds, playing a significant role in the intestinal microbiome. *Alistipes* is associated with protective effects against conditions like liver fibrosis and colitis, but also with pathogenic roles in colorectal cancer and mental health disorders [67]. This widespread presence across breeds indicates a fundamental impact on intestinal health, influenced by genetic and environmental factors.

Detection of *Clavispora* and *Blastocystis* across all breeds with healthy subjects suggests their potential role as stable components of the gut microbiota, likely influenced by environmental factors [68]. Known for stimulating mucus production through IL-22 and promoting bacterial diversity, *Blastocystis* may have beneficial impacts on intestinal health and immune function [69]. Pichia emerged as one of the most abundant genera in healthy cattle breeds, suggesting its beneficial role in their gut microbiota. Although Pichia has been associated with higher body mass index (BMI) in humans [68], its presence in healthy cattle and alpacas points to a species-specific role in supporting intestinal health without adverse effects [70]. Additionally, Trichostomatia was identified as a prevalent genus in cattle, pigs [44], and other bovines [43], underscoring its significant role in the intestinal microbiota. This aligns with findings of *Trichostomatia* as a diverse and notable component of the gut microbiota in patients with healthcare-associated diarrhea [71]. These consistent observations across different species and health conditions suggest that Trichostomatia plays a crucial role in maintaining a varied and resilient intestinal ecosystem.

A significant negative correlation was observed between the genus *Blautia* and hip skin thickness (GPC). Similarly, in a study on cattle, *Blautia* wexlerae exhibited a negative correlation with backfat thickness (BFT) [45]. Additionally, decreased abundance of *Blautia* wexlerae was associated with reduced levels of acetic and butyric acids, essential for lipid metabolism and adiposity traits [72,73]. In the same study, *Coprococcus* also demonstrated a negative correlation with meat quality parameters, similar to this study where a negative correlation was observed with the gluteus medius muscle area (NGM) in both NGM1 and NGM2. Likewise, *Christensenellaceae R-7* presented significant negative correlations [74] with GPC and loin area (AL). These consistent correlations across different studies [74,75] underscore the potential role of these genera in lipid metabolism and adiposity traits in cattle, suggesting they could influence fat deposition and meat quality. This knowledge is crucial for developing targeted strategies to improve meat quality through modulation of gut microbiota composition. Dietary supplementation with *Rhodotorula mucilaginosa* has been demonstrated to significantly improve all color parameters of chicken meat, highlighting its potential to optimize the visual quality of meat products [76]. In this study, *Rhodotorula* exhibited a positive correlation with the gluteus medius muscle area (NGM) in both NGM1 and NGM2, and a negative correlation with the thickness of the loin skin, suggesting a role in reducing adiposity and improving muscle composition. The presence of *Rhodotorula* sp. in the gut could positively influence meat quality by producing beneficial nutrients such as proteins, lipids, folate, and carotenoids [77]. Additionally, its

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probiotic effect, which regulates the multiplication of pathogenic bacteria and neutralizes their toxins [78], could improve intestinal health and, consequently, meat quality.

Cryptosporidium, a genus of protists, has been linked to significant negative correlations with several meat quality traits in cattle, including AL, GL, and GPC. This relationship may be due to its impact on the intestinal microbiota, which is crucial for nutrient absorption and metabolism. Studies have demonstrated that the composition of the gut microbiota significantly influences meat quality by affecting metabolic processes and nutrient utilization [79,80]. Therefore, the presence of *Cryptosporidium* could negatively affect these processes, leading to poorer meat quality characteristics, such as reduced muscle mass and increased fat deposition [81,82]. Understanding these interactions is vital to developing strategies to mitigate the negative effects of *Cryptosporidium* on meat quality through targeted microbiota management and improved animal health practices.

In Angus cattle, Christensenellaceae has been identified as a biomarker associated with lean body mass and metabolic health, suggesting its influence on fat deposition and overall meat quality [83]. In Braunvieh cattle, the genus *Dorea* was identified, known for its ability to regulate intestinal health and nutrient absorption, playing a crucial role in the gut microbiome [84,85]. In Simmental cattle, *Eubacterium coprostanoligenes* was identified. Members of this genus are known for their butyrate production, which plays a critical role in energy homeostasis, colonic motility, immunomodulation, and suppression of gut inflammation. Additionally, *Eubacterium* is involved in bile acid and cholesterol transformation, contributing to their homeostasis [86,87]. Gut dysbiosis and altered representation of *Eubacterium* in the gut have been associated with various disease states in humans [88].

The Spearman correlation analysis reveals intricate relationships between various alpha diversity indices and hematological and meat quality parameters, highlighting the multifaceted interactions within the gut microbiota and host physiological traits. For bacterial alpha diversity, significant positive correlations were observed between the Shannon index and GPL, suggesting that greater bacterial diversity might be associated with better skin condition [89]. Conversely, negative correlations of the Simpson and Pielou indices with NGM2 marbling indicate a potential link between bacterial evenness and marbling traits. These findings align with previous studies emphasizing the influence of gut microbiota diversity on animal health and meat quality [45,84].

Significant correlations between the observed fungal index and blood parameters such as MON% and EOS suggest an interaction between fungal diversity and immune cell populations. Negative correlations with GL, AL and GPC also indicate that greater fungal diversity could influence fat deposition and distribution. This is consistent with previous studies highlighting the role of fungal communities in nutrient metabolism and immune modulation [90]. Furthermore, hematological health can affect the intestinal mycobiota, crucial for understanding how variations in hematological parameters influence the diversity and functionality of the intestinal mycobiota [91]. For protists, positive correlations of alpha diversity indices with PLT and negative correlations with growth and loin parameters (WG, GL, AL, GPC) underscore the complex role of protists in intestinal health and animal growth [92,93].

The correlation analysis indicated that several meat quality parameters are significantly correlated with the beta diversity of fungi and protists. These correlations align with recent studies demonstrating that the gut microbiota is influenced by host genetic factors [94]. For example, the composition of the rumen microbiota and the function of certain microorganisms are determined by host genetics and can influence feed efficiency and meat quality [74]. Studies have identified genomic regions associated with the abundance of certain rumen bacteria, suggesting that genetic selection could be used to improve ruminal function and feed efficiency in cattle [95].

The approach to control for age-related variations limited the sample size and the proportion of sexes of Simmental, Brahman, and crossbred cattle available for the study. The cattle's age was uniform to eliminate the influence of this factor in group comparisons.

5. Conclusions

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In this study, significant correlations were observed between gut microbiota diversity and various hematological and meat quality parameters across different cattle breeds. These results emphasize the intricate relationship between the gut microbiome and host physiological traits, suggesting that microbial diversity plays a vital role in influencing health and production traits. The findings indicate that specific microbial communities within the gut are linked to better skin condition, fat deposition, and muscle quality, highlighting the potential for targeted microbiota management. Notably, a limitation of the study was the control for age-related variations, which reduced the sample size and the proportion of sexes of Simmental, Brahman, and crossbred cattle available for analysis. The study underscores the importance of considering genetic and environmental factors, including breed, in shaping the gut microbiota. By understanding these interactions, strategies can be developed to optimize gut health, enhance feed efficiency, and improve meat quality in livestock. Future research should further explore these relationships to refine cattle management and breeding practices, ultimately contributing to the advancement of livestock production systems.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1: Rarefaction curves of species richness show the sequencing depth of data obtained from the gut samples. Figure S2: Alpha diversity metrics of protists were evaluated in cattle in five breeds. Diversity indices exhibited include ACE, Chao1, Observe, Pielou, Shannon and Simpson. Figure S3. The Venn diagram based on ASVs of cattle fecal microbiota from the five breeds. Figure S4. Relative abundances in the gut microbiota at the phylum and genus level in different cattle breeds. Table S1: List of abbreviations. Table S2: Variables of five breeds groups. Table S3: Kruskal Wallis results for clinical parameters in cattle by breed. Table S4: Posthoc Bonferroni results for variables in cattle by breed.

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