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# From association to intervention: *Muribaculaceae* driven SCFAs production enhances boar semen quality via inflammation alleviation

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## Abstract

The gut microbiota plays a vital role in host reproduction, yet its contribution to semen quality in boars remains unclear. In this study, we analyzed 556 boars from three commercial breeds and identified *Muribaculaceae* as a key microbial taxon positively associated with sperm quality, with the effect mediated by short-chain fatty acids (SCFAs). This association was validated in Yorkshire boars with extreme semen phenotypes. Fecal microbiota transplantation in mice confirmed that enrichment of *Muribaculaceae* improved semen quality, likely through enhanced SCFA production and reduced inflammation in the gut and reproductive tract. Furthermore, *in vitro* fermentation and mouse experiments demonstrated that a designed functional fiber selectively promoted *Muribaculaceae*, increased SCFA levels, and improved sperm quality. These findings suggest a functionally supported and potentially translational association between gut microbiota and boar fertility, suggesting that targeted dietary modulation of *Muribaculaceae* may represent a novel strategy to enhance reproductive performance in livestock.

## Introduction

Male fertility is essential for ensuring the efficiency and sustainability of livestock breeding systems. In boars, semen quality is a key determinant of reproductive performance, with poor semen traits being one of the leading causes of premature culling[1]. Although host genetics contribute to variation in semen quality[2,3], emerging evidence highlights the gut microbiota as a crucial and potentially modifiable factor influencing reproductive health[4,5]. Although the role of gut microbiota in male fertility has been extensively studied in humans and mice[6-8], research in boars remains limited, and the microbial mechanisms underlying semen quality variation in livestock species are still poorly understood. Given the high reproductive value of boars and the economic importance of semen quality, investigating the role of gut microbiota in boar fertility is both scientifically valuable and practically relevant.

Previous studies have shown that gut microbiota composition varies significantly among pig breeds[9,10], and that semen quality traits also exhibit breed-specific differences[2,3], suggesting that breed-specific gut microbes may be closely linked to reproductive performance. Gut microbiota can influence host reproductive function through several mechanisms, including the conversion of dietary nutrients into short-chain fatty acids (SCFAs)[11], maintenance of intestinal barrier integrity[6], modulation of systemic immune and inflammatory responses[12], and regulation of sex hormone levels[13]. However, systematic efforts to identify specific microbes associated with reproductive traits in boars and to clarify their causal roles remain scarce. Meanwhile, growing evidence suggests that gut microbiota is highly responsive to dietary components, particularly fermentable fibers, which can selectively promote the growth of specific bacterial taxa[14-16]. This raises the possibility that targeted dietary interventions may modulate gut microbial composition and metabolic activity, thereby affecting host reproductive outcomes.

In light of the limited understanding of key microbes and their modifiability, this study employed a multi-step approach to systematically identify gut microbial taxa associated with semen quality and evaluate their potential as targets for nutritional intervention (Fig. 1). First, using a large cohort of boars from three commercial breeds, we integrated gut microbiota profiles, SCFA measurements, and semen quality traits to identify *Muribaculaceae* as a key taxon positively associated with sperm performance. Second, within the Yorkshire breed, we validated this

association in animals with extreme semen phenotypes, confirming the intra-breed relevance of *Muribaculaceae*. Third, fecal microbiota transplantation (FMT) from *Muribaculaceae*-rich donors improved semen quality in recipient mice, likely by increasing SCFA levels and reducing inflammation. Fourth, *in vitro* fermentation was used to screen for dietary fibers that promote *Muribaculaceae,* and their efficacy was validated in a mouse model, where fiber supplementation increased *Muribaculaceae* abundance and SCFA levels, thereby improving sperm quality. Together, these four steps outline a mechanistic and translational pathway from microbial association to nutritional intervention, offering novel insights into how targeted modulation of the gut microbiota, particularly *Muribaculaceae*, may improve male fertility in livestock.

## Results

**Boar semen quality exhibits breed-specific variations.**

We first presented the semen quality traits according to breed (Table 1). Semen volume differed significantly among breeds, following the pattern of Duroc < Landrace ≈ Yorkshire ($P < 0.001$). In contrast, sperm concentration and abnormal sperm rate were significantly higher in Duroc boars than in Landrace and Yorkshire boars ($P < 0.001$). Sperm motility exhibited a trend toward significance among breeds ($P = 0.052$), with the highest values observed in Yorkshire boars.

**Table 1 Differences in semen quality of different breeds of boars**

| Traits | Duroc (n=175) | Landrace (n=195) | Yorkshire (n=186) | *P* value |
|---|---|---|---|---|
| Semen volume, mL | $171.19 \pm 50.16^{B}$ | $201.27 \pm 53.06^{A}$ | $200.76 \pm 68.65^{A}$ | < 0.001 |
| Sperm concentration, $\times 10^{8}$ $mL^{-1}$ | $5.11 \pm 1.70^{A}$ | $4.05 \pm 1.32^{B}$ | $3.68 \pm 1.21^{B}$ | < 0.001 |
| Sperm motility, % | 88.85[86.50-90.58] | 88.31[85.27-90.55] | 89.26[85.83-91.63] | 0.052 |
| Abnormal sperm rate, % | $6.85[4.50\text{-}10.09]^{A}$ | $4.25[3.00\text{-}6.69]^{B}$ | $3.81[2.62\text{-}6.43]^{B}$ | < 0.001 |

The results are presented as the mean ± standard deviation for normal distribution or median [one quarter-three quarters] for skewed distribution. In the same row, values bearing distinct uppercase superscripts signify a significant difference ($P < 0.01$).

**Breed is a major determinant of gut microbiota composition in boars**

The gut bacterial profiles of 556 boars were analyzed using amplicon sequencing (Fig. 2A). Upon initial analysis, an average of 55,347 quality-filtered bacterial sequences were generated per boar. After denoising and clustering, a total of 45,232 ASVs were identified and assigned to various taxonomic levels, encompassing 42 phyla, 96 classes, 217 orders, 399 families, and 1040 genera within the bacterial communities. PERMANOVA was performed to determine the factors contributing to gut microbiota variation in boars. Specifically, breed was the predominant factor in the multivariate model, explaining approximately 7.5% of the variation, followed by age ($R^2$ = 0.0340). Additionally, housing unit ($P$ = 0.0010), sampling month ($P$ = 0.0006), and medication ($P$ = 0.0008) also significantly affected the composition of boars' gut microbiota (Fig. 2B). Given that breed was identified as the dominant factor influencing gut microbial variation, we next compared the diversity and composition of gut microbiota among the three boar breeds. Alpha diversity analysis showed that the Chao1 richness index was significantly lower in Duroc boars compared to Landrace ($P$ = 0.004) and Yorkshire boars ($P$ = 4.3e-5) (Fig. 2C). The Shannon index showed a trend toward lower diversity in Duroc boars compared to Yorkshire boars ($P$ = 0.052; Fig. 2D). Principal coordinate analysis (PCoA) based on Bray-Curtis distances revealed clear separation among the three breeds, primarily along the PCo2 axis (Fig. 2E). Notably, the greatest separation was observed between Duroc and Yorkshire boars. Relative abundances of gut bacterial taxa at the phylum and genus levels are shown in Fig. 2F and Fig. 2G, respectively, highlighting the top 10 most abundant taxa in each group. Further analysis of differential abundance using the linear discriminant analysis effect size (LEfSe) approach identified bacterial taxa specific to each breed. For instance, *Lactobacillus* and *Terrisporobacter* were significantly enriched in Duroc boars, *Clostridium_sensu_stricto_1* and *Ruminococcaceae_UCG-005* were predominant in Landrace boars, whereas *Christensenellaceae_R-7_group* and *Muribaculaceae* were enriched in Yorkshire boars (Fig. 2H).

**Mediation analysis highlights the potential role of SCFAs in linking gut microbiota to semen quality**

To further explore the functional implications of breed-associated gut microbiota, we employed PICRUSt2 to infer microbial functions based on 16S rRNA gene sequences, referencing the MetaCyc database. Under the primary classification category “Generation of Precursor Metabolite

and Energy" the secondary category "fermentation" emerged as the most abundant (Fig. 3A). At the third-level classification, the pathways involved in the fermentation of propionate and butyrate were significantly more enriched in the gut microbiota of Landrace and Yorkshire boars compared to Duroc boars ($P < 0.05$, Fig. S1). Targeted metabolite profiling of fecal SCFAs revealed that acetate, propionate, and butyrate were the predominant SCFAs in boar feces, while valerate, isobutyrate, and isovalerate were detected at lower levels. Yorkshire boars exhibited significantly higher fecal acetate levels than Duroc boars ($P < 0.05$; Fig. 3B). Notably, propionate and butyrate levels followed a descending trend: Yorkshire > Landrace > Duroc ($P < 0.001$; Fig. 3B).

We next conducted pairwise Spearman correlation analyses among semen quality traits, fecal bacterial genera, and SCFAs concentrations (Fig. 3C). Bacterial genera enriched in Yorkshire boars, such as *Christensenellaceae_R-7_group* and *Muribaculaceae*, were positively correlated with SCFAs levels and negatively correlated with abnormal sperm rate. Furthermore, SCFAs concentrations were positively associated with sperm motility and negatively associated with abnormal sperm rate. To assess whether SCFAs mediated the effects of gut microbiota on semen quality, we performed 224 mediation analyses across 14 differential genera, 4 SCFAs, and 4 semen quality traits. Nineteen significant mediation pathways were identified, involving six bacterial genera affecting three semen quality traits through two SCFAs (Fig. 3D). Notably, *Muribaculaceae* was found to enhance sperm motility via butyrate production ($P_{medi} = 0.030$) and reduced abnormal sperm rate through increased propionate levels ($P_{medi} = 0.048$; Fig. 3E).



### *Muribaculaceae* enhances semen quality by promoting the production of SCFAs and mitigating inflammation

Based on our large-scale, multi-breed analysis, *Muribaculaceae* was identified as a potentially beneficial genus that promotes SCFA production and improves semen quality. Given its highest relative abundance in the gut microbiota of Yorkshire boars, we selected phenotypically extreme individuals from this breed to further explore the mechanisms through which *Muribaculaceae* influences semen quality (Fig. 4A). Significant differences in semen quality traits were observed between the high-quality (H) and low-quality (L) groups, particularly in the sperm abnormality rate ($P < 0.001$; Fig. 4B). β-diversity analysis revealed distinct gut microbial compositions between

the two groups (Fig. 4C). *Muribaculaceae*, *Prevotellaceae_NK3B31_group*, and *Rikenellaceae_RC9_gut_group* were enriched in the H group, whereas *Streptococcus* and *Oscillospira* were enriched in the L group (Fig. 4D). Correlation analyses showed that *Muribaculaceae* and *Prevotellaceae_NK3B31_group* were positively associated with sperm motility and negatively with abnormal sperm rate, whereas *Streptococcus* exhibited the opposite pattern (Fig. 4E). Fecal SCFA analysis indicated a trend toward higher propionate levels ($P$ = 0.064) and significantly higher butyrate levels ($P < 0.05$) in the H group compared to the L group (Fig. 4F). Furthermore, serum analysis revealed significantly elevated testosterone levels in the H group ($P < 0.05$; Fig. 4G). Additionally, boars in the L group exhibited significantly higher levels of serum endotoxin, IL-6, and TNF-α, along with lower IL-10 levels ($P < 0.05$; Fig. 4H), suggesting a heightened inflammatory state potentially associated with reduced semen quality.

**FMT confirms the role of *Muribaculaceae* in improving semen quality through SCFAs production**

To investigate the causal effect of *Muribaculaceae* on semen quality, we conducted an FMT experiment in mice using donor feces from Yorkshire boars with either extremely high or low *Muribaculaceae* abundance, which was associated with significant differences in semen quality (Fig. 5A). After 12 weeks of FMT, no significant differences in body weight were observed among the groups (Fig. S2A). At the end of the experiment, mice in the HFMT group exhibited significantly higher sperm motility than those in the CON ($P < 0.01$) and LFMT groups ($P < 0.001$). Conversely, the abnormal sperm rate was significantly lower in the HFMT group than in the LFMT group ($P < 0.001$), with a decreasing trend compared to the CON group ($P$ = 0.063; Fig. 5B). Additionally, serum testosterone levels were significantly higher in the HFMT group compared with the other two groups ($P < 0.05$; Fig. 5C). H&E staining of testicular tissue revealed disorganization of the seminiferous epithelium in the LFMT group, characterized by exfoliated germ cells in the lumen, whereas such abnormalities were absent in the CON and HFMT groups (Fig. 5D). The morphology of the epididymal tissues did not differ significantly among the groups (Fig. 5E).

Analysis of fecal microbiota revealed no significant differences in α-diversity among the three groups (Fig. S2B), whereas β-diversity showed significant variation (Fig. 5F). At the phylum level, LFMT mice exhibited a higher relative abundance of Firmicutes and a lower abundance of

Bacteroidota compared with the other groups (Fig. S2C). At the genus level, *Muribaculaceae* was significantly enriched in both HFMT and CON mice relative to LFMT mice (Fig. S2C). LEfSe analysis further confirmed the significantly higher abundance of *Muribaculaceae* in HFMT mice compared to LFMT mice (Fig. 5G). Notably, fecal concentrations of acetate, propionate, butyrate, and total SCFAs were significantly higher in the HFMT group than in the LFMT group (Fig. 5H).

SCFAs play diverse physiological roles in the host, contributing to intestinal homeostasis, regulating systemic inflammation, and supporting the function of distant organs. To further investigate how SCFAs mediate the beneficial effects of a *Muribaculaceae*-enriched microbiota on reproductive performance, we examined inflammatory responses in the intestinal tract and reproductive organs of FMT recipient mice. Mice in the LFMT group showed significantly lower villus-to-crypt ratios in both the jejunum and ileum compared to the CON and HFMT groups (Fig. 6A-C, Fig. S2D-E).

Additionally, histological analysis revealed marked inflammatory cell infiltration in the colonic tissues of LFMT mice (Fig. 6D). qPCR analysis of colonic tight junction gene expression showed that *ZO-1* was significantly upregulated in the HFMT group compared to the LFMT mice ($P < 0.05$; Fig. 6E). *ZO-2* expression also showed an increasing trend in the CON ($P = 0.078$) and HFMT ($P = 0.083$) groups relative to the LFMT group. No significant differences were found in the expression of *Occludin* or *Claudin-4* among the groups. Immunofluorescence staining for M1 macrophage markers revealed a higher pro-inflammatory macrophage signal in the colons of LFMT mice (Fig. 6F). Furthermore, the colonic expression of inflammatory cytokines *IL-6* and *TNF-α* was significantly lower in HFMT mice compared to LFMT mice ($P < 0.05$; Fig. 6G).

We further analyzed inflammatory gene expression in the testes and epididymides. Compared to HFMT mice, LFMT mice showed significantly higher testicular expression of *IL-6*, *IL-1β*, and *TNF-α* ($P < 0.05$; Fig. 6H). In the epididymis, *IL-1β* and *TNF-α* were also significantly upregulated in LFMT mice compared to HFMT mice ($P < 0.05$; Fig. 6I).

**Dietary fiber-induced enrichment of *Muribaculaceae* enhances semen quality in mice**

After confirming the beneficial effects of *Muribaculaceae*-enriched fecal microbiota transplantation on semen quality, we sought to identify dietary fibers capable of selectively promoting *Muribaculaceae* growth as a feasible nutritional strategy.

*In vitro* fermentation was performed using fecal microbiota from high-quality (H) and low-quality (L) Yorkshire boars as inocula, with three candidate dietary fibers resistant starch (RS), inulin (IN), and a composite functional fiber (FF) used as substrates (Fig. 7A). After 48 hours of fermentation, the fluids inoculated with L-group microbiota exhibited significantly lower pH values under the IN and FF conditions compared to the blank control ($P < 0.01$; Fig. S3A). qPCR analysis showed that the relative gene copy number of *Muribaculaceae* was highest in the LFF group, significantly surpassing those in the L, LRS, and LIN groups ($P < 0.01$). *Prevotellaceae* gene copy numbers were similarly elevated in both the LIN and LFF groups ($P < 0.01$), while *Streptococcus* levels were significantly reduced in these groups compared to the L and LRS groups ($P < 0.01$; Fig. 7B). SCFA analysis revealed that the LFF fermentation fluid contained the highest levels of acetate, butyrate, and total SCFAs, along with significantly more propionate than the L and LIN groups ($P < 0.01$; Fig. 7C). Similar trends were observed in the H-group fermentation, where the HFF condition resulted in the lowest pH, highest *Muribaculaceae* abundance, and the greatest total SCFA production ($P < 0.01$; Fig. S3B-C). Based on these findings, FF was selected as the optimal dietary fiber for enriching *Muribaculaceae*.

To assess its *in vivo* efficacy, three treatment groups were established: PBS-ND (control), LFMT-ND (LFMT with a normal diet), and LFMT-FF (LFMT with an FF-supplemented diet) (Fig. 7D). After six weeks of intervention, qPCR analysis indicated a trend toward reduced fecal *Muribaculaceae* abundance in LFMT-ND mice compared to PBS-ND ($P = 0.073$), whereas FF supplementation significantly restored its levels in LFMT-FF mice ($P < 0.01$; Fig. 7E). No significant differences in *Prevotellaceae* gene copy number were observed among the groups. However, *Streptococcus* levels were significantly elevated in LFMT-ND mice and reduced by FF supplementation ($P < 0.05$; Fig. 7E). Analysis of fecal SCFAs indicated that acetate ($P = 0.096$), butyrate ($P = 0.089$), and total SCFA levels ($P = 0.070$) were slightly lower in LFMT-ND mice than in PBS-ND mice. In contrast, all SCFA concentrations were significantly increased in LFMT-FF mice compared to LFMT-ND mice ($P < 0.05$; Fig. 7F). Regarding semen quality, LFMT-ND mice showed significantly higher rates of abnormal sperm compared to PBS-ND controls ($P < 0.05$), with no significant differences in other parameters (Fig. 7G). Compared to LFMT-ND mice, FF supplementation (LFMT-FF) significantly enhanced sperm motility ($P < 0.05$), tended to

increase total sperm count ($P$ = 0.091), and markedly reduced the rate of abnormal sperm ($P$ < 0.05; Fig. 7G).

## Discussion

Although the role of the gut microbiota in host reproduction is increasingly recognized, its specific impact on semen quality in livestock remains poorly understood. In this study, we conducted a large-scale analysis involving 556 boars from three commercial breeds and identified *Muribaculaceae* as a key microbial taxon associated with semen quality traits. Moreover, by targeting key microbial taxa through dietary fiber intervention, we offer novel insights into enhancing boar fertility through microbiota modulation.

The composition of the gut microbiota varies considerably across different boar breeds[9,10,17]. While environmental factors, particularly diet, are often considered the primary determinants of gut microbiota composition[14], a growing body of evidence suggests that host genetics also plays a critical role in shaping the pig gut microbial community[9,18,19]. This genetic influence becomes particularly evident under standardized conditions, where dietary and environmental variables are controlled. In addition to influencing specific bacterial taxa, host genetics may also impact overall gut microbial diversity[20,21]. For instance, we observed significantly greater microbial richness in Yorkshire boars compared to Duroc boars, whereas evenness did not differ significantly among breeds, consistent with previous findings[9,17]. These observations may be partly explained by differences in host genetic background. Previous studies have reported that gut microbial diversity indices in pigs are influenced by host genetics and exhibit moderate heritability[20,21], indicating that genetic factors contribute to shaping the gut microbial community. In commercial breeding programs, Duroc pigs are predominantly selected as terminal sires, whereas Landrace and Yorkshire pigs, or their crossbred offspring, are typically used as maternal lines. The distinct breeding objectives for paternal and maternal lines, such as emphasis on growth performance and carcass traits in sire lines versus reproductive performance and robustness in maternal lines, may impose different selective pressures on host physiology and metabolism, thereby indirectly influencing gut microbial diversity and community structure[9].

The gut microbiota exerts broad physiological effects on the host via its functional activities. Functional profiling of the microbiota revealed significant enrichment in fermentation-associated

metabolic pathways. This is consistent with the ecological strategy of gut microbes, which rely on the fermentation of undigested carbohydrates to support their growth[22,23]. These carbohydrates are fermented into short-chain fatty acids (SCFAs), which play vital roles in gut health maintenance and systemic metabolic regulation. Fecal SCFA concentrations were significantly higher in Yorkshire boars compared to Duroc boars, potentially reflecting differences in microbial composition between breeds. In Yorkshire boars, the gut microbiota was enriched with *Muribaculaceae*, *Christensenellaceae_R-7_group*, and *Ruminococcaceae_UCG-002* taxa well known for their fiber-degrading capabilities and SCFA production, particularly propionate and butyrate[24-28]. In contrast, *Lactobacillus*, *Terrisporobacter*, and *Rikenellaceae_RC9_gut_group* were more abundant in Duroc boars, taxa typically linked to lactate and acetate production[29,30]. These taxonomic patterns corroborate our functional predictions based on 16S rRNA gene sequencing and underscore breed-specific differences in microbial fermentation capacity.

Mediation analysis revealed that *Muribaculaceae*, *Rikenellaceae_RC9_gut_group*, and *Treponema_2* may contribute to reduced sperm abnormality rates in boars through propionate. However, *Rikenellaceae_RC9_gut_group* was not significantly correlated with the phenotype, and the abundance of *Treponema_2* was relatively low. Therefore, *Muribaculaceae* was identified as the most likely key taxon influencing semen quality. *Muribaculaceae*, formerly known as family S24-7, is a bacterial family characterized by its broad repertoire of carbohydrate-degrading enzymes[24]. It is considered a primary fiber-degrading taxon capable of breaking down dietary fibers into soluble forms that can be further utilized by secondary degraders[31]. A previous genomic analysis of 153 *Muribaculaceae* strains revealed an abundance of carbohydrate-active enzymes, particularly glycoside hydrolase families GH13 and GH43, suggesting their role in degrading starch, arabinoxylan, and xylan[32]. Consistent with previous studies[31,33], we also observed a significant association between *Muribaculaceae* and gut propionate concentrations, further supporting the role of short-chain fatty acids, particularly propionate, in enhancing boar semen quality.

FMT is a well-established method for validating causal relationships between specific microbes and host phenotypes[5,6]. In our study, we found that increasing the relative abundance of *Muribaculaceae* in the gut via FMT elevated SCFA production in mice, ultimately leading to improved semen quality. These findings suggest that targeted modulation of *Muribaculaceae* may

offer a promising strategy for improving boar semen quality. Given that *Muribaculaceae* is a well-known fiber-degrading taxon, we aimed to increase its abundance through dietary fiber intervention. Based on literature review and preliminary experiments, we selected several fiber types known to promote *Muribaculaceae* growth, including resistant starch, inulin[31], and a proprietary functional fiber blend previously developed in our laboratory[34]. Among these, the functional fiber exhibited the strongest effect in promoting *Muribaculaceae* proliferation, increasing SCFA production, and improving semen quality in mice. The superior performance of the functional fiber may be attributed to two factors: first, it may better match the substrate preferences of *Muribaculaceae*; second, the diversity of its fiber components may create a more favorable and versatile environment for *Muribaculaceae* enrichment and activity.

In addition to serving as key microbial metabolites, SCFAs, particularly propionate and butyrate, are essential for maintaining intestinal homeostasis by strengthening the intestinal barrier, modulating immune responses, and inhibiting pathogenic bacteria[35]. Several studies have demonstrated that elevated concentrations of SCFAs in the gut are positively correlated with enhanced semen quality across multiple species, including pigs[11], chickens[36], and mice[8]. One potential mechanism underlying this association is the anti-inflammatory effect of SCFAs. Both intestinal and reproductive tract inflammation can impair spermatogenesis and lead to increased sperm abnormalities. Elevated SCFA levels may reduce systemic or local inflammation by promoting regulatory T cell differentiation and suppressing the expression of pro-inflammatory cytokines, thereby creating a more favorable environment for normal sperm development. These findings suggest that SCFA-mediated modulation of host inflammation may be a key mechanism through which the gut microbiota affects semen quality.

Despite providing novel insights into the relationship between *Muribaculaceae*, SCFA production, and semen quality, this study has several limitations. First, we relied primarily on 16S rRNA gene sequencing to characterize the gut microbiota, which limits taxonomic resolution and precludes functional gene analysis. A metagenomic approach would have provided deeper insights into microbial functions and metabolic pathways. Second, although *Muribaculaceae* was identified as a potentially beneficial taxon, we did not isolate and validate its effects using mono-colonization or gnotobiotic models, which are crucial for establishing causal relationships. Third, all functional validations were performed in mice. While these findings are promising, they require further

validation in pigs, the target species, to ensure their translatability and practical relevance to swine production.

In summary, our study identifies *Muribaculaceae* as a key gut microbial taxon associated with a lower sperm deformity rate, likely through increased propionate production. FMT and fiber intervention experiments in mice further support its functional role in improving semen quality. These findings highlight the potential of targeting *Muribaculaceae* as a novel microbial strategy to enhance reproductive traits in livestock, laying the groundwork for future applications in pigs.

## Methods

### Boar sample collection

A total of 556 boars from three breeds, including purebred Duroc (D, n = 175), Landrace (L, n = 195), and Yorkshire (Y, n = 186), were raised under uniform management at an artificial insemination station located in southern China. These boars ranged in age from 8 to 69 months and were housed in six separate barns. The rearing conditions followed those described previously[37]. Fresh fecal samples were collected from each boar by rectal massage, immediately flash-frozen in liquid nitrogen, and stored at -80 ℃ until analysis (Fig. 2A). Blood samples were collected from the hindlimb veins and centrifuged at 3000 r/min for 10 minutes to separate the serum, which was subsequently stored at -20 ℃. All animal research procedures were conducted according to the animal research guidelines issued by the Institutional Animal Care and Use Committee of Huazhong Agricultural University (permit number HZAUSW-2020-0008 and 202402240012).

Semen was collected approximately every five days, and semen quality was assessed at each collection. Recorded parameters included semen volume, sperm concentration, sperm motility, and abnormal sperm rate, all measured using previously established methods[37]. To minimize individual testing variation, the average value for each semen parameter was calculated based on data collected within one month before and one month after the sampling date (Fig. 2A).

### 16S rRNA sequencing

Fecal DNA was separately extracted from boars and mice using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany), and the quality of each sample was subsequently assessed. Amplification

of the V3-V4 regions of the 16S rRNA gene was performed with forward primer 341F (ACTCCTACGGGAGGCAGCA) and reverse primer 806R (GGACTACHVGGGTWTCTAAT). The amplification products were purified by magnetic beads (Vazyme VAHTSTM) and subjected to fluorescence quantification (BioTek, FLx800) to adjust the sample ratio. Sequencing libraries were then prepared using Illumina's TruSeq Nano DNA LT Library Prep Kit, and bipartite sequencing was conducted using a NovaSeq sequencer (Shanghai Personal Biotechnology Co., Ltd.). The downstream sequencing data underwent quality control procedures and followed the DADA2 (2019.4) analysis pipeline in QIIME2 for sequence clustering[38]. The amplicon sequence variant (ASV) taxonomy was determined by a BLAST search against a representative set of sequences in the Silva database (v132)[39].

**Short-chain fatty acid detection**

The concentration of short-chain fatty acids in feces was measured by gas chromatography according to previously described protocols[37]. Briefly, approximately 0.1 g of fecal sample was mixed with phosphate-buffered saline (PBS) at a ratio of 1:9, vortexed thoroughly, and subjected to low-speed centrifugation. The resulting supernatant was combined with 25% phosphoric acid at a ratio of 5:1, mixed well, and incubated overnight at 4 °C. On the following day, the sample was centrifuged at high speed, and the supernatant was extracted with an equal volume of ethyl acetate. After a second high-speed centrifugation, the supernatant was filtered through a 0.22 μm organic membrane filter and transferred into gas chromatography vials, which were stored at -20 °C until analysis. SCFAs were quantified using a Thermo Trace 1300 gas chromatograph equipped with a TG-WAXMSA column (Thermo Scientific, #26087-1420), and concentrations were determined based on standard calibration curves generated from known SCFA standards. In this study, the sum of acetate, propionate, and butyrate concentrations was defined as total SCFAs, while the combined concentrations of isobutyrate and isovalerate were defined as total branched-chain fatty acids.

**Mediation analysis**

Mediation analysis was conducted using the mediate function in the R package mediation (version 4.5.0) to explore whether fecal short-chain fatty acids (SCFAs) mediated the association between gut microbial taxa (predictor, x) and semen quality parameters (outcome, y). Two separate linear models were fitted: one modeling the mediator as a function of the microbial abundance and age

(SCFA ~ x + age), and the other modeling the outcome as a function of both the microbial predictor and the mediator, again adjusting for age (y ~ x + SCFA + age). To estimate the indirect (mediated) and direct effects, nonparametric bootstrapping was employed with 1,000 iterations to derive 95% confidence intervals.

**FMT experiment**

Fecal bacterial suspensions were prepared according to a previously reported protocol[5]. Fecal samples from three randomly selected boars in each of the H group and L group were mixed separately. A 20% sterile glycerol PBS was used as the diluent, and the fecal bacterial suspensions were prepared at a dilution ratio of 1:10. The mixtures were vortexed thoroughly and filtered through a 70 μm filter. The filtrate containing the bacteria was collected, aliquoted, and stored at -80 °C until use. Before FMT, the frozen fecal suspensions were gently thawed in a 37°C water bath. After thawing, the suspensions were diluted with sterile saline to a standard concentration of $1 \times 10^9$ colony-forming units (CFU)/mL. A total of 24 male ICR mice aged 6 weeks were randomly assigned to three groups: the CON group (gavage with 20% sterile glycerol PBS), the HFMT group, and the LFMT group, with eight mice per group. Mice in each group were orally gavaged with 200 μL of the corresponding suspension once every two days for 12 consecutive weeks. At the end of the experiment, mice were anesthetized using ether by exposure to ether-soaked sterile degreased cotton placed in a transparent anesthesia bag until loss of consciousness, after which samples were collected according to the experimental design.

**ELISA analysis**

The concentrations of serum-related substances were measured using pig ELISA kits for testosterone (MM-0410O2), endotoxin (MM-36368O2), interleukin-6 (IL-6, MM-0418O2), interleukin-10 (IL-10, MM-0425O1), and tumor necrosis factor-α (TNF-α, MM-0383O2), as well as a mouse testosterone ELISA kit (MM-0569M1), all purchased from Jiangsu Meimian Industrial Co., Ltd. The assays were performed according to the manufacturer's instructions.

**Sperm quality assessment in mice**

Based on our previous study, sperm quality was assessed through sperm count, motility, morphology, and membrane integrity[40]. Briefly, one side of the epididymis was placed in 1 mL of prewarmed saline (37 °C), minced, and incubated for 20 min to obtain a sperm suspension. For

sperm concentration, 10 μL of suspension was loaded onto a prewarmed hemocytometer, and sperm were counted in five large squares using standard red blood cell counting methods. To assess sperm motility, another 10 μL drop was examined under a light microscope at 37 °C. The percentage of progressively motile sperm (moving in a straight line) was calculated from a total of 200 sperm. For sperm morphology, 10 μL of the suspension was smeared, air-dried, fixed with methanol (15 min), stained with 2% eosin (1 h), rinsed, and dried. At least 200 sperm per sample were analyzed under high magnification. Abnormalities in the head, midpiece, tail, or presence of cytoplasmic droplets were counted. Membrane integrity was evaluated using the hypoosmotic swelling test with another 100 μL aliquot. All measurements were performed in duplicates, and the mean values were used for analysis.

**Morphology and Immunofluorescence**

Intestinal, testicular, and epididymal tissues were quickly collected and fixed in 4% paraformaldehyde, then transferred to 70% ethanol. Samples were dehydrated and embedded in paraffin, and serial sections of 4 μm thickness were prepared. After deparaffinization, the sections were subjected to hematoxylin and eosin (H&E) staining for histological analysis. For immunofluorescence, sections were washed and underwent antigen retrieval. They were then incubated in 3% hydrogen peroxide solution in the dark for 20 minutes. After washing with PBS three times, 100 μL of primary antibody against CD86 (CST, Cat#: 19589, 1:400 dilution) was added, and the sections were incubated overnight at 4°C. The next day, the primary antibody was removed, and sections were washed three times in PBS. Subsequently, a secondary antibody (SeraCare, Cat#: 5220-0336, 1:400 dilution) was applied for 1 hour, followed by DAPI staining for 10 minutes. After another three PBS washes in the dark, 5 μL of anti-fade mounting medium was added to each sample during slide preparation. Cover slips were placed, and slides were stored at 4°C in the dark. Fluorescent images were captured using a fluorescence microscope.

**Real-time quantitative PCR analyses**

Total RNA from tissue samples was isolated using TRIzol reagent (Vazyme, Cat#: R40101). RNA concentration and purity were assessed with a NanoDrop ND-1000 spectrophotometer (Thermo, Waltham, USA), and reverse transcription was performed using a commercial reverse transcriptase kit (ABclonal, Cat#: RK20433) to synthesize cDNA. Quantitative real-time PCR (qPCR) was conducted on a Bio-Rad real-time PCR system (California, USA) with SYBR Green Master Mix

(Vazyme, Cat#: Q321) to measure mRNA expression levels. For bacterial DNA analysis, genomic DNA was extracted from fermentation fluid and fecal samples using the TIANGEN fecal DNA extraction kit (Cat#: DP328-02), followed by quantification with the NanoDrop ND-1000. Quantitative PCR for bacterial abundance was performed on the Bio-Rad CFX96 system. Primer sequences used for gene and bacterial quantification are provided in Table S1.

### *In vitro* fecal fermentation

The dietary fiber substrates used in this experiment included inulin (IN, Shanghai Aladdin Biochemical Technology Co., Ltd., China), resistant starch (RS, Shandong Yunzhou Science and Technology Co., Ltd., China), and a functional fiber (FF) developed by our research group[34,41]. FF is composed of 14.3% guar gum (Shandong Yunzhou Science and Technology Co., Ltd.) and 85.7% pregelatinized waxy maize starch (Zhejiang Puluoxiang Starch Co., Ltd.). *In vitro* digestion of the fiber substrates was performed based on a modified version of the method previously reported by Noack et al.[42,43]. Briefly, fiber samples were dissolved in PBS and mixed thoroughly before adding α-amylase solution. After further mixing, concentrated HCl was added to adjust the pH to 2.0, followed by the addition of pepsin solution (1 mg/mL). After stirring, the pH was adjusted to 6.9 using concentrated NaOH solution. Finally, 5 mL of pancreatin solution was added, and the mixture was stirred for 180 minutes. The digestion residues were collected, freeze-dried using a freeze dryer (Germany Chris Co., Germany), and stored at -20°C for subsequent fermentation.

The fermentation inocula were prepared by first mixing fecal samples from three randomly selected boars from the H group, and separately from the L group. Each pooled fecal sample was then processed under anaerobic conditions by adding sterile saline at a ratio of 1:5 (w/v). The mixture was thoroughly stirred and filtered through four layers of gauze. The resulting filtrate served as the fermentation inoculum for the corresponding group. For *in vitro* fermentation, each sample was processed in triplicate. A total of 0.35 g of freeze-dried enzymatic digestion residue was added to a 150 mL fermentation bottle, followed by 56.55 mL of nutrient solution (composed of 52.41 mL basal solution, 0.69 mL vitamin-phosphate solution, 2.76 mL sodium bicarbonate buffer, and 0.69 mL reducing agent) and 3.45 mL of fermentation inoculum. Fermentation was conducted at 39°C.

**Functional fiber feeding trial**

Eighteen six-week-old male ICR mice were randomly divided into three groups (n = 6 per group): PBS-ND, LFMT-ND, and LFMT-FF. Mice in the PBS-ND group received oral gavage of PBS and were fed a normal diet. The LFMT-ND group received FMT from Yorkshire boars with low semen quality and were also fed a normal diet. The LFMT-FF group received the same FMT treatment and was fed a diet in which 6% functional fiber replaced the cellulose in the normal diet. The detailed composition of the diets is provided in Table S2. FMT was performed once every two days and continued for 45 days. All mice were housed under controlled temperature conditions (21-22 °C) with a 12-hour light/dark cycle.

**Statistical analysis**

Factors impacting the genus-level composition of boar gut microbiota were examined using permutational multivariate analysis of variance (PERMANOVA) with the adonis function in R software (version 4.4.2). The analysis included six factors: boar breed (Duroc, Landrace, and Yorkshire), age (in months), herd type (A to C indicates a gradual decrease in genetic quality), medication (whether antibiotics were administered to the boar in the two months before sampling), month of sampling (October, November, December, and January), and housing unit (unit 1 to unit 6). Alpha diversity within the gut microbiota and the content of fecal SCFAs across different boar breeds were assessed using the Kruskal-Wallis rank-sum test, followed by Dunn's test as a post hoc analysis to confirm the significance of observed variations. The results of Spearman's correlation analysis were presented by heatmap plotting using the R software pheatmap package (v1.0.12). Some of the statistical analyses and figures were generated using GraphPad Prism version 9.5. Student’s t-test was used to compare differences between two groups. For comparisons among three or more groups, one-way ANOVA was applied, followed by Tukey’s multiple comparison test when appropriate. Results are expressed as mean ± standard deviation. A $P$-value < 0.05 was considered statistically significant. Significance levels are indicated as follows: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

## Data availability

Boar fecal 16S rRNA gene sequencing data are available from the Sequence Read Archive under accession number PRJNA1007937. Mouse fecal 16S rRNA gene sequencing data are available from the Genome Sequence Archive under accession number CRA028648.

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**Fig. 1: Experimental design.**

Step 1: Identification of key microbe *Muribaculaceae* across three pig breeds. Step 2: Validation in Yorkshire boars. Step 3: Causal relationship tested via FMT. Step 4: Fiber screening and intervention targeting *Muribaculaceae*.

**Fig. 2: Breed-specific differences in gut microbiota composition of boars.**

**A** Schematic diagram of the experimental design involving three boar breeds. **B** Bar plot of PERMANOVA results showing factors contributing to gut microbial variation. **C-D** Boxplots of Chao1 index (**C**) and Shannon index (**D**). **E** PCoA plot based on Bray-Curtis distances showing microbial community separation by breed. **F-G** Relative abundance of bacterial taxa at the phylum (**F**) and genus (**G**) levels (top 10 most abundant taxa shown). **H** Differentially enriched genera among breeds identified by LEfSe analysis (linear discriminant analysis score > 3.0). The asterisks denote statistically significant differences: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

**Fig. 3: SCFAs mediate the effects of gut microbiota on semen quality.**

**A** Functional prediction of boar gut microbiota. **B** Boxplot of fecal SCFAs concentrations among breeds. BCFAs: branched-chain fatty acids. **C** Spearman correlations among bacterial genera, SCFAs, and semen quality traits. **D** Mediation analysis of genus-SCFAs-semen quality traits pathways. **E** *Muribaculaceae* reduced the abnormal sperm rate via propionate production. A plus sign (+) indicates a positive effect; a minus sign (–) indicates a negative effect. Asterisks indicate statistical significance: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

**Fig. 4: *Muribaculaceae*-associated gut profile supports better semen quality in Yorkshire boars.**

**A** Schematic of experimental design based on extreme semen quality in Yorkshire boars. **B** Comparison of semen quality traits between high-quality (H) and low-quality (L) groups. **C** PCoA showing gut microbial differences between H and L groups. **D** Differentially abundant genera between groups identified by LEfSe (linear discriminant analysis score > 3.0). **E** Spearman

correlations among genera and semen quality traits. **F** Fecal SCFAs concentrations in H and L groups. **G** Serum testosterone levels. **H** Serum levels of endotoxin, IL-6, TNF-α, and IL-10. All values are shown as mean ± SD. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

**Fig. 5: *Muribaculaceae*-enriched fecal microbiota improves semen quality in mice.**

**A** Schematic of the FMT experiment. **B** Semen phenotypes of recipient mice. **C** Serum testosterone levels in recipient mice. **D-E** H&E staining of testis (**D**) and epididymis (**E**) tissues. **F** Gut microbial differences among groups based on PCoA. **G** Differentially abundant genera identified by LEfSe. **H** Fecal concentrations of SCFAs. All values are shown as mean ± SD. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

**Fig. 6: Histological changes and inflammatory status in intestinal and reproductive tissues of FMT recipient mice.**

**A-B** H&E staining of jejunum (**A**) and ileum (**B**). **C** Quantification of villus-to-crypt ratio. **D** H&E staining of colon tissue. **E** mRNA expression of tight junction proteins in the colon. **F** Immunofluorescence staining of M1 macrophage markers in colon. **G-I** mRNA expression of inflammatory cytokines in colon (**G**), testis (**H**), and epididymis (**I**). All values are shown as mean ± SD. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

**Fig. 7: Screening and validation of a *Muribaculaceae*-targeting fiber.**

**A** Schematic of *in vitro* fermentation using fecal microbiota from low-quality (L) boars and three dietary fibers. **B** qPCR analysis of bacterial gene expression in L-group fermentation fluid. **C** SCFAs concentrations in L-group fermentation fluid. **D** Schematic of an in vivo experiment in FMT recipient mice. **E** Fecal gene expression of target bacteria. **F** Fecal SCFAs concentrations. **G** Semen quality parameters in mice. All values are shown as mean ± SD. Different uppercase letters above bars indicate *P* < 0.01. **P* < 0.05, ***P* < 0.01.

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### Contributions

J.P. conceived and designed the experiments and wrote and revised the manuscript. H.K.W. designed the experiments and revised the manuscript. L.L.G. performed the experiments, collected the samples, analyzed the data, and wrote the manuscript. X.Q.P. conducted the mouse

experiments, collected samples and performed the tests. J.J.T. and H.Q.S. collected the samples. S.W.J. analyzed the data. All the authors have read and approved the final manuscript.

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## Ethics declarations

**Competing interests**

The authors declare no competing interests.

## Additional information

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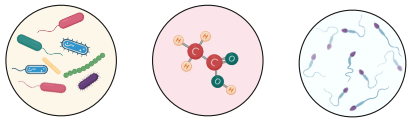
## Supplementary Information

Supplementary Information

1.

Large cohort of three breeds(n=556)



Microbiota–SCFAs–Semen quality

Key microbe
Muribaculaceae

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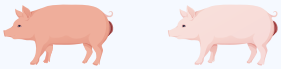
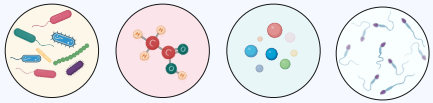


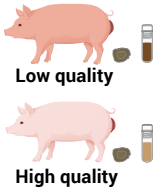
Yorkshire boars
low Vs. high semen quality



Gut microbiota - SCFAs - Cytokines - Semen quality

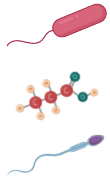
Key microbe
Muribaculaceae

3.



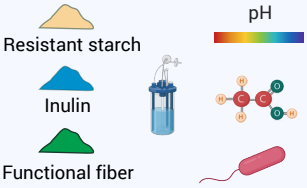

Fecal microbiota transplantation


Exploration of potential mechanisms

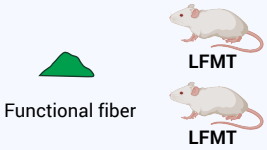


Muribaculaceae-SCFAs-Sperm

4.

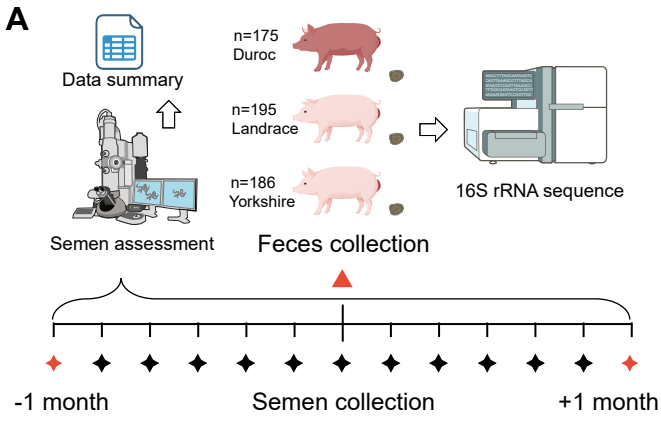



Screening of fibers targeting
the regulation of Muribaculaceae




Functional fiber feeding trial in mice

Improved semen quality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Landrace
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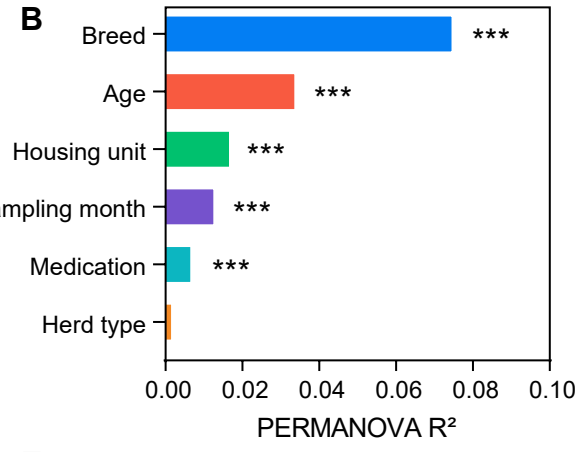
Yorkshire
16S rRNA sequence
Semen assessment
Feces collection
-1 month
Semen collection
+1 month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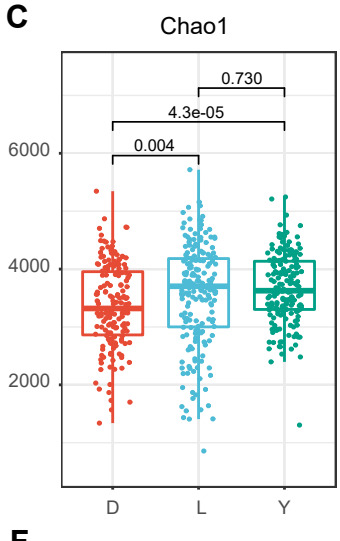
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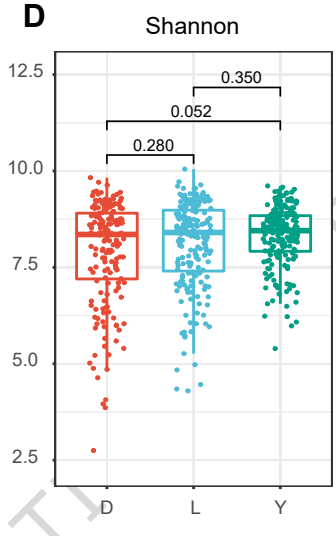
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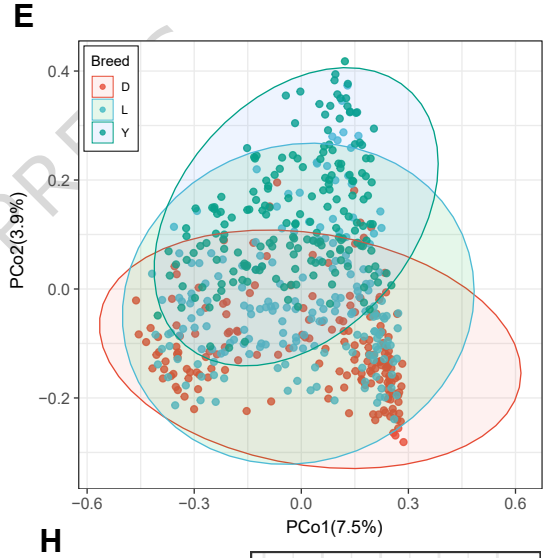
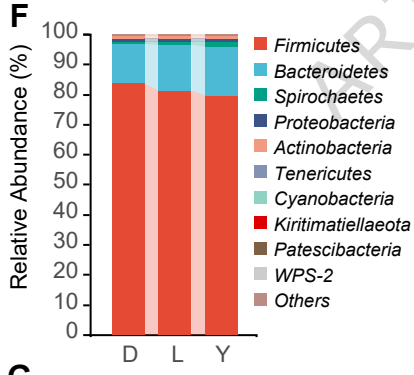
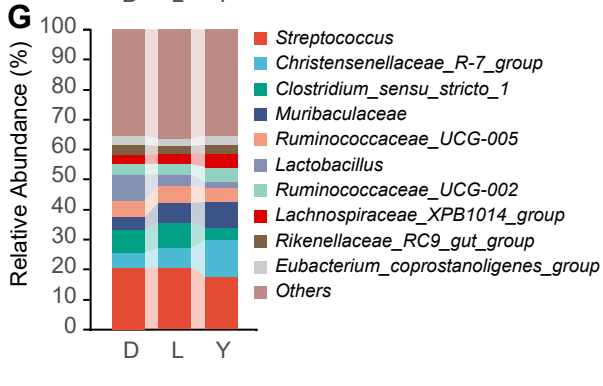
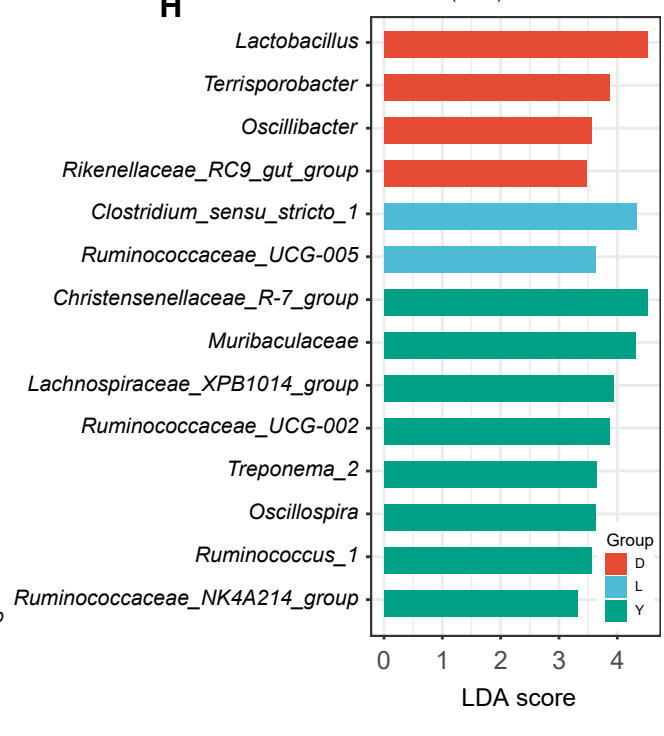
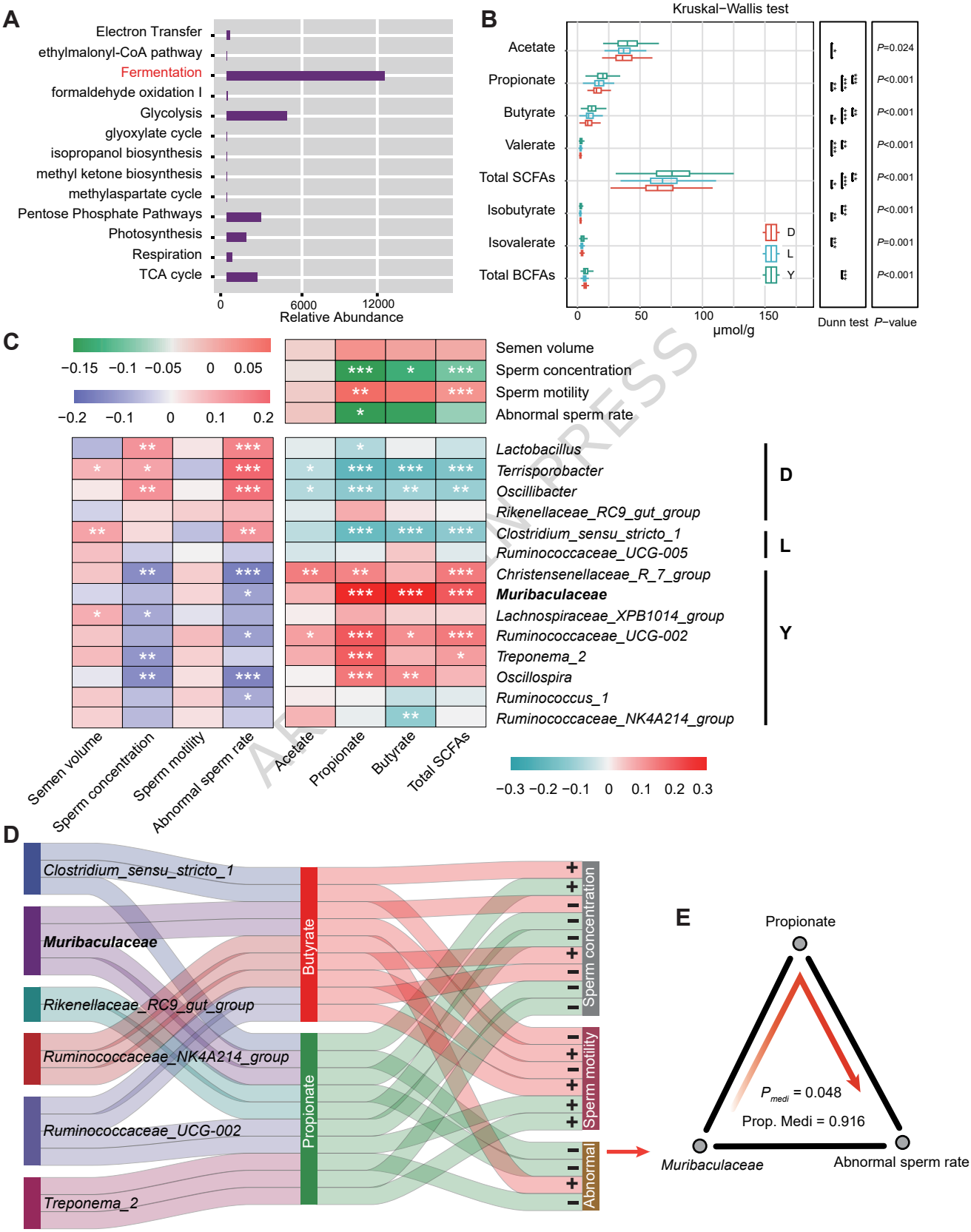
L
Y

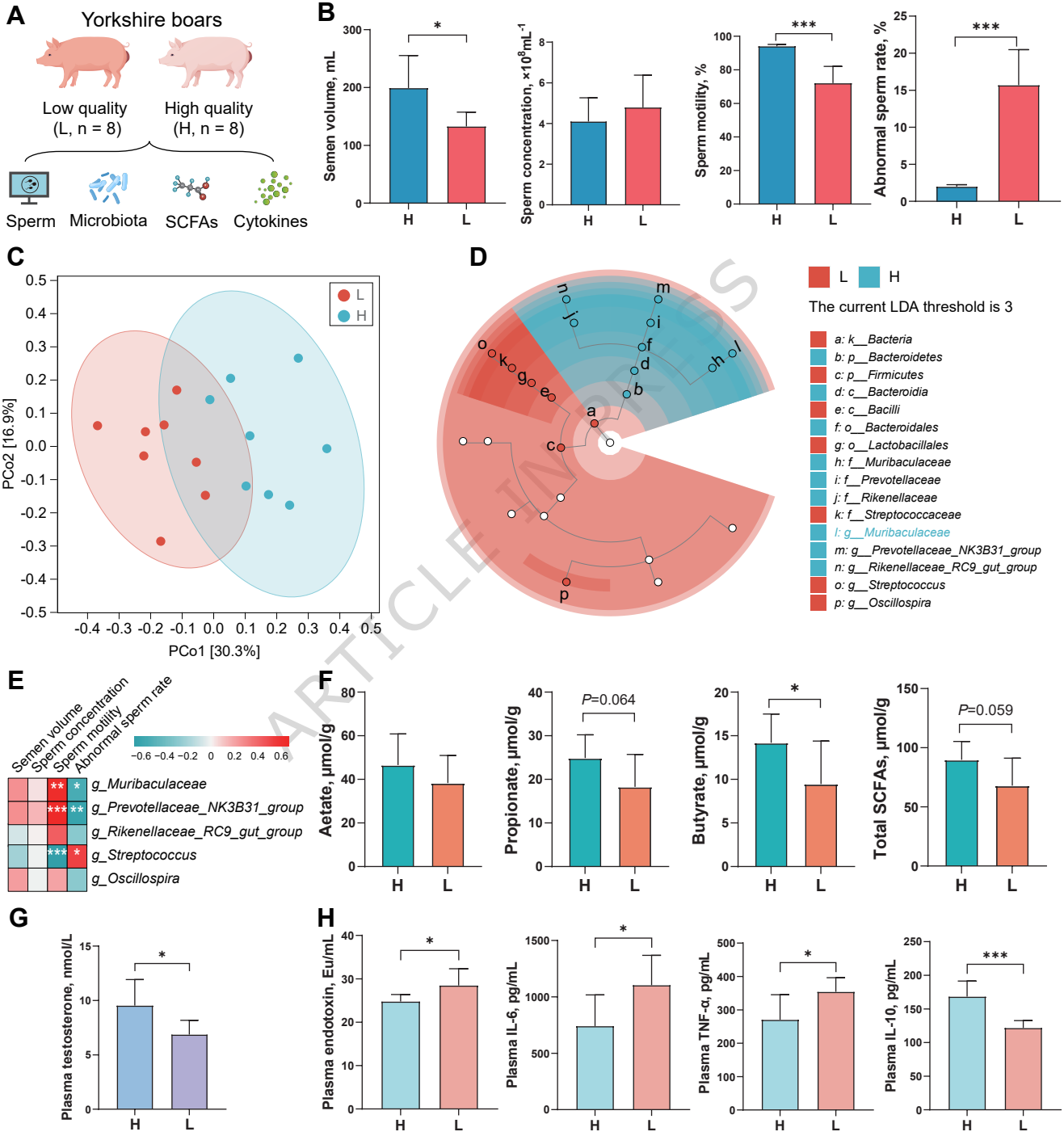

E
Breed
D
L
Y
PCo2(3.9%)
PCo1(7.5%)

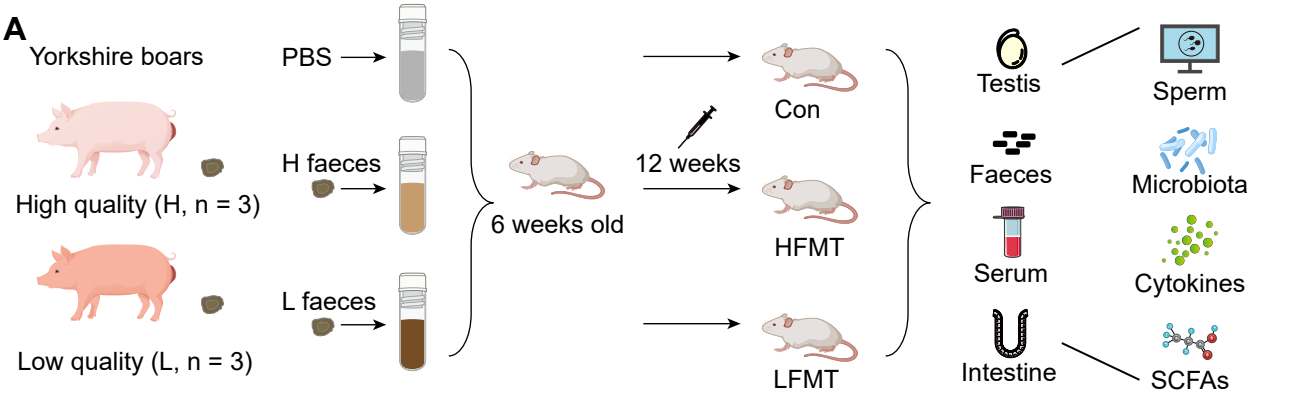

F
Relative Abundance (%)
Firmicutes
Bacteroidetes
Spirochaetes
Proteobacteria
Actinobacteria
Tenericutes
Cyanobacteria
Kiritimatiellaeota
Patescibacteria
WPS-2
Others
D
L
Y

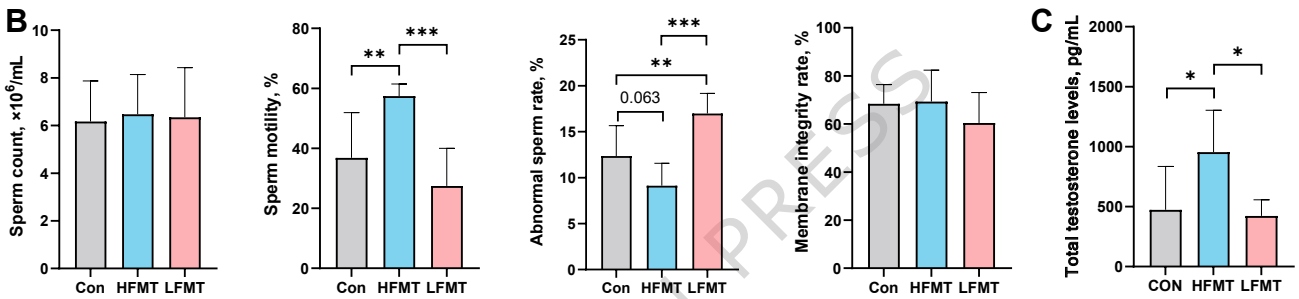

G
Relative Abundance (%)
Streptococcus
Christensenellaceae_R-7_group
Clostridium_sensu_stricto_1
Muribaculaceae
Ruminococcaceae_UCG-005
Lactobacillus
Ruminococcaceae_UCG-002
Lachnospiraceae_XPB1014_group
Rikenellaceae_RC9_gut_group
Eubacterium_coprostanoligenes_group
Others
D
L
Y

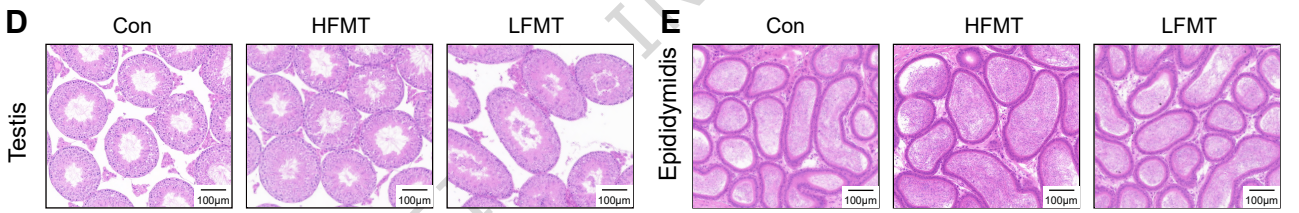

H
Lactobacillus
Terrisporobacter
Oscillibacter
Rikenellaceae_RC9_gut_group
Clostridium_sensu_stricto_1
Ruminococcaceae_UCG-005
Christensenellaceae_R-7_group
Muribaculaceae
Lachnospiraceae_XPB1014_group
Ruminococcaceae_UCG-002
Treponema_2
Oscillospira
Ruminococcus_1
Ruminococcaceae_NK4A214_group
Group
D
L
Y
LDA score

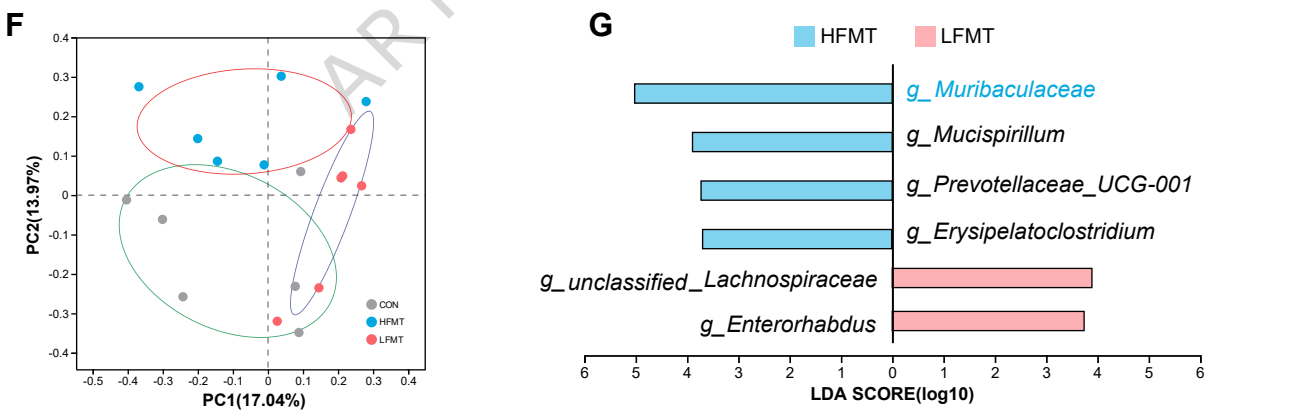

A
Electron Transfer
ethylmalonyl-CoA pathway
Fermentation
formaldehyde oxidation I
Glycolysis
glyoxylate cycle
isopropanol biosynthesis
methyl ketone biosynthesis
methylaspartate cycle
Pentose Phosphate Pathways
Photosynthesis
Respiration
TCA cycle
0
6000
12000
Relative Abundance
B
Kruskal−Wallis test
Acetate
Propionate
Butyrate
Valerate
Total SCFAs
Isobutyrate
Isovalerate
Total BCFAs
D
L
Y
0
50
100
150
μmol/g
Dunn test
P−value
P=0.024
P<0.001
P<0.001
P<0.001
P<0.001
P<0.001
P=0.001
P<0.001
C
−0.15 −0.1 −0.05 0 0.05
−0.2 −0.1 0 0.1 0.2
Semen volume
Sperm concentration
Sperm motility
Abnormal sperm rate
Lactobacillus
Terrisporobacter
Oscillibacter
Rikenellaceae_RC9_gut_group
Clostridium_sensu_stricto_1
Ruminococcaceae_UCG-005
Christensenellaceae_R_7_group
Muribaculaceae
Lachnospiraceae_XPB1014_group
Ruminococcaceae_UCG-002
Treponema_2
Oscillospira
Ruminococcus_1
Ruminococcaceae_NK4A214_group
D
L
Y
Semen volume
Sperm concentration
Sperm motility
Abnormal sperm rate
Acetate
Propionate
Butyrate
Total SCFAs
−0.3 −0.2 −0.1 0 0.1 0.2 0.3
D
Clostridium_sensu_stricto_1
Muribaculaceae
Rikenellaceae_RC9_gut_group
Ruminococcaceae_NK4A214_group
Ruminococcaceae_UCG-002
Treponema_2
Butyrate
Propionate
Sperm concentration
Sperm motility
Abnormal
E
Propionate
P_medi = 0.048
Prop. Medi = 0.916
Muribaculaceae
Abnormal sperm rate

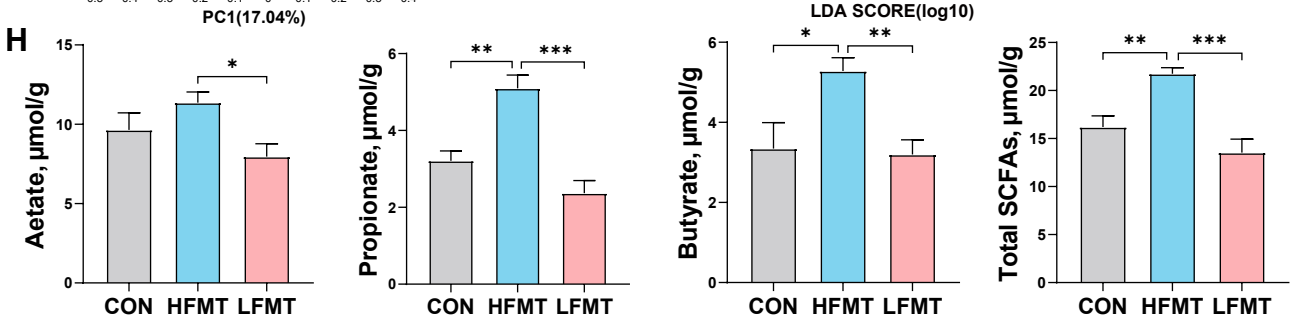

A
Yorkshire boars
Low quality
(L, n = 8)
High quality
(H, n = 8)
Sperm
Microbiota
SCFAs
Cytokines
B
Semen volume, mL
Sperm concentration, ×10^8 mL^-1
Sperm motility, %
Abnormal sperm rate, %
H
L
C
PCo2 [16.9%]
PCo1 [30.3%]
D
The current LDA threshold is 3
a: k__Bacteria
b: p__Bacteroidetes
c: p__Firmicutes
d: c__Bacteroidia
e: c__Bacilli
f: o__Bacteroidales
g: o__Lactobacillales
h: f__Muribaculaceae
i: f__Prevotellaceae
j: f__Rikenellaceae
k: f__Streptococcaceae
l: g__Muribaculaceae
m: g__Prevotellaceae_NK3B31_group
n: g__Rikenellaceae_RC9_gut_group
o: g__Streptococcus
p: g__Oscillospira
E
Semen volume
Sperm concentration
Sperm motility
Abnormal sperm rate
g_Muribaculaceae
g_Prevotellaceae_NK3B31_group
g_Rikenellaceae_RC9_gut_group
g_Streptococcus
g_Oscillospira
F
Aetate, μmol/g
Propionate, μmol/g
P=0.064
Butyrate, μmol/g
Total SCFAs, μmol/g
P=0.059
G
Plasma testosterone, nmol/L
H
Plasma endotoxin, Eu/mL
Plasma IL-6, pg/mL
Plasma TNF-α, pg/mL
Plasma IL-10, pg/mL

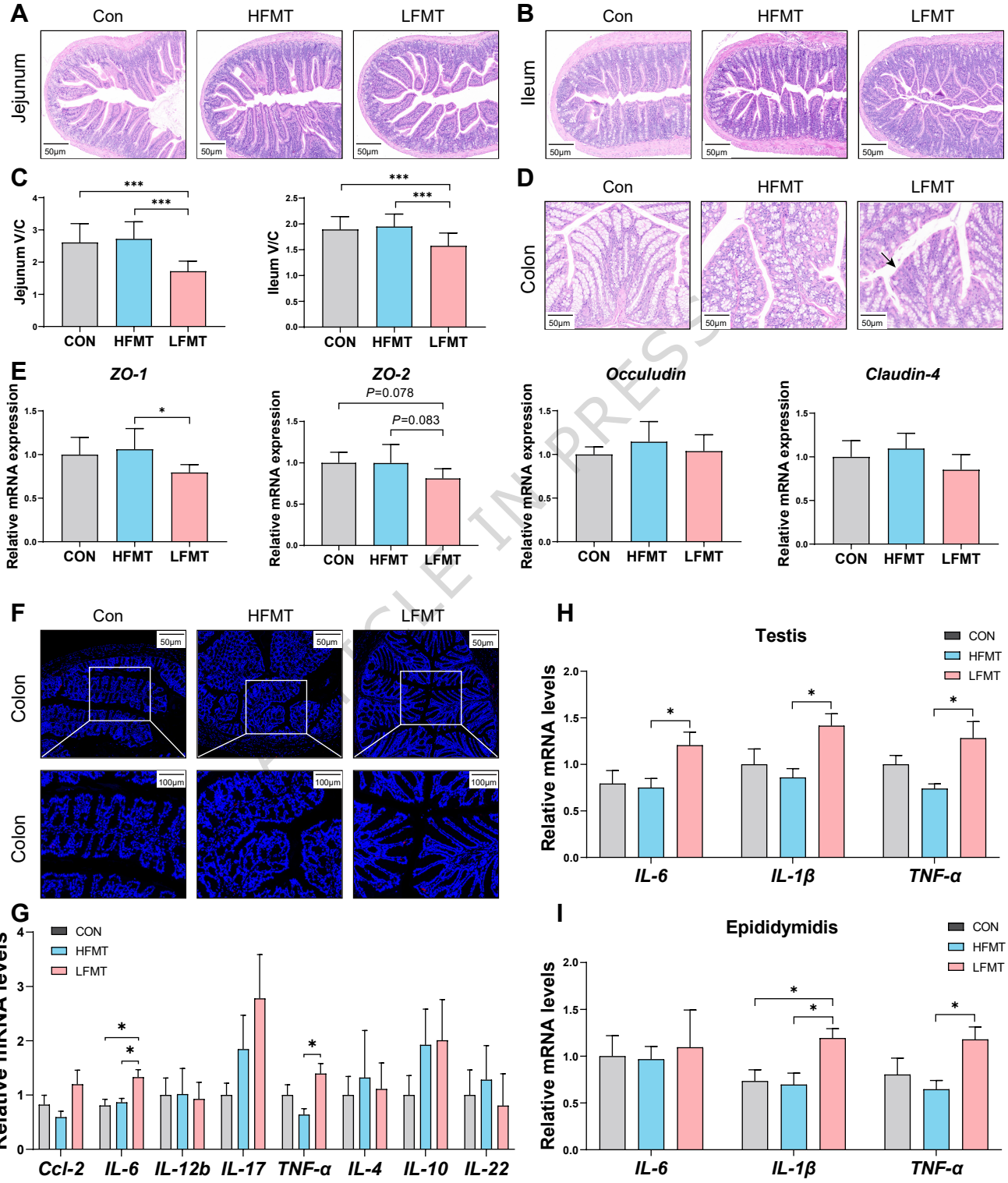

A
Yorkshire boars
High quality (H, n = 3)
Low quality (L, n = 3)
PBS
H faeces
L faeces
6 weeks old
12 weeks
Con
HFMT
LFMT
Testis
Faeces
Serum
Intestine
Sperm
Microbiota
Cytokines
SCFAs

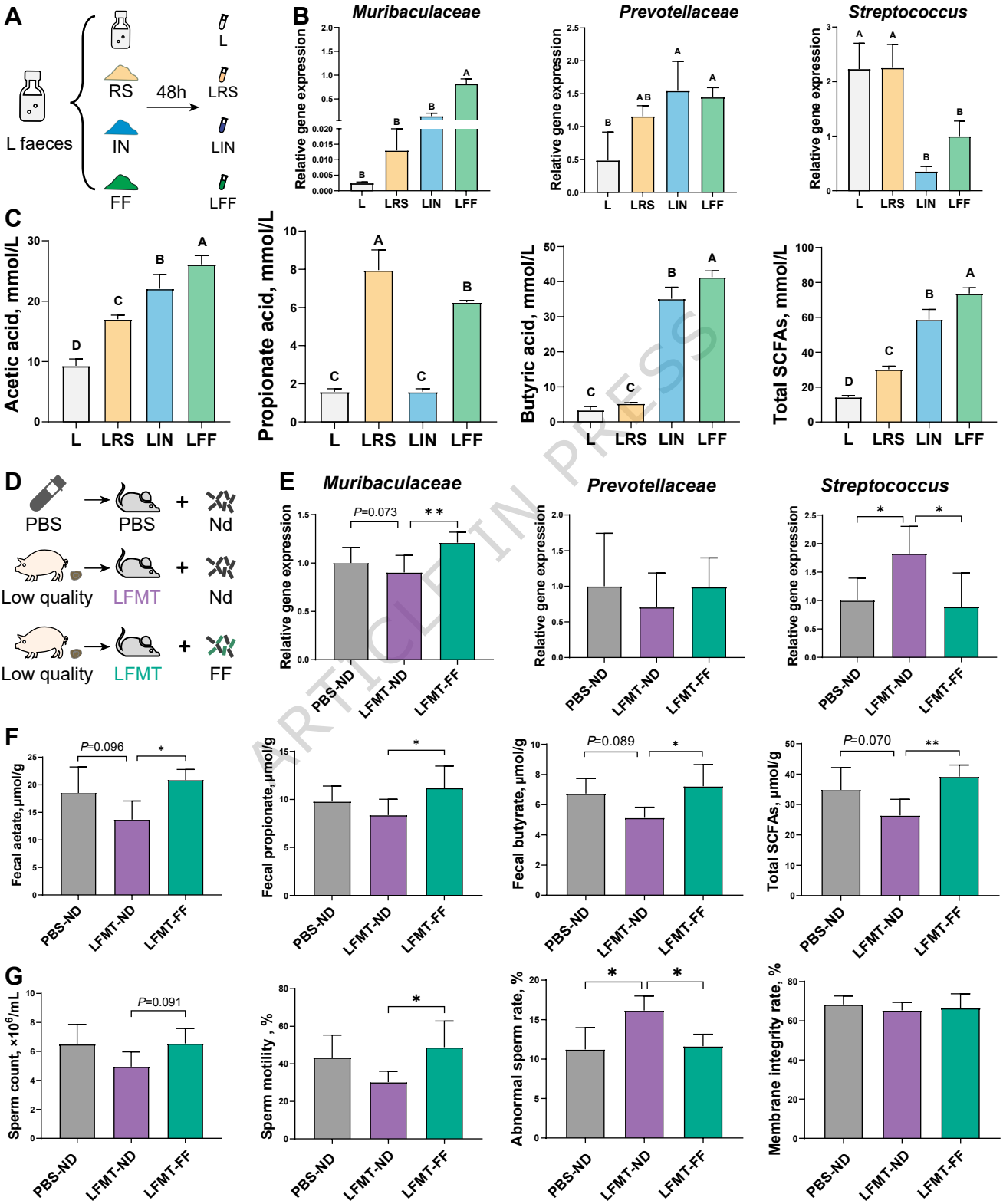

B
Sperm count, ×10⁶/mL
Sperm motility, %
Abnormal sperm rate, %
0.063
Membrane integrity rate, %
Con HFMT LFMT
C
Total testosterone levels, pg/mL
CON HFMT LFMT


D
Con
HFMT
LFMT
Testis
100μm
E
Epididymidis


F
PC2(13.97%)
PC1(17.04%)
CON
HFMT
LFMT
G
HFMT
LFMT
g_Muribaculaceae
g_Mucispirillum
g_Prevotellaceae_UCG-001
g_Erysipelatoclostridium
g_unclassified_Lachnospiraceae
g_Enterorhabdus
LDA SCORE(log10)


H
Aetate, μmol/g
Propionate, μmol/g
Butyrate, μmol/g
Total SCFAs, μmol/g
CON HFMT LFMT




A
L faeces
RS
IN
FF
48h
L
LRS
LIN
LFF
B
Muribaculaceae
Prevotellaceae
Streptococcus
Relative gene expression
C
Acetic acid, mmol/L
Propionate acid, mmol/L
Butyric acid, mmol/L
Total SCFAs, mmol/L
D
PBS
PBS
Nd
Low quality
LFMT
Nd
Low quality
LFMT
FF
E
Muribaculaceae
Prevotellaceae
Streptococcus
P=0.073
PBS-ND
LFMT-ND
LFMT-FF
F
Fecal aetate, μmol/g
P=0.096
Fecal propionate, μmol/g
Fecal butyrate, μmol/g
P=0.089
Total SCFAs, μmol/g
P=0.070
G
Sperm count, ×10^6/mL
P=0.091
Sperm motility, %
Abnormal sperm rate, %
Membrane integrity rate, %