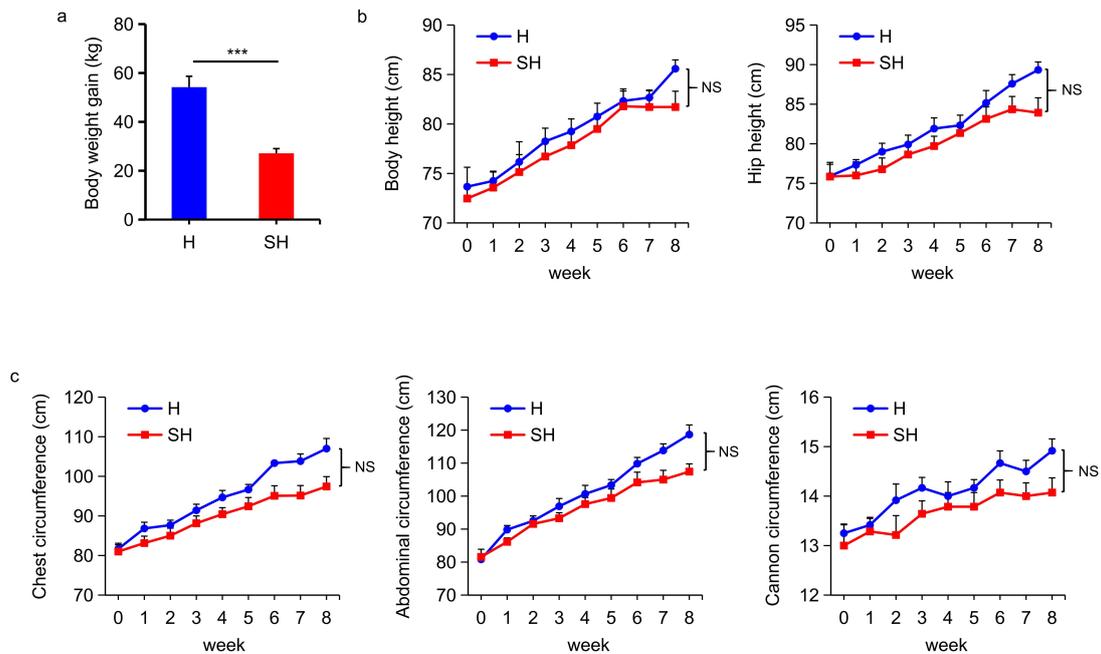


Dynamic changes of intestinal microbiota and metabolite composition in pre-weaned beef calves

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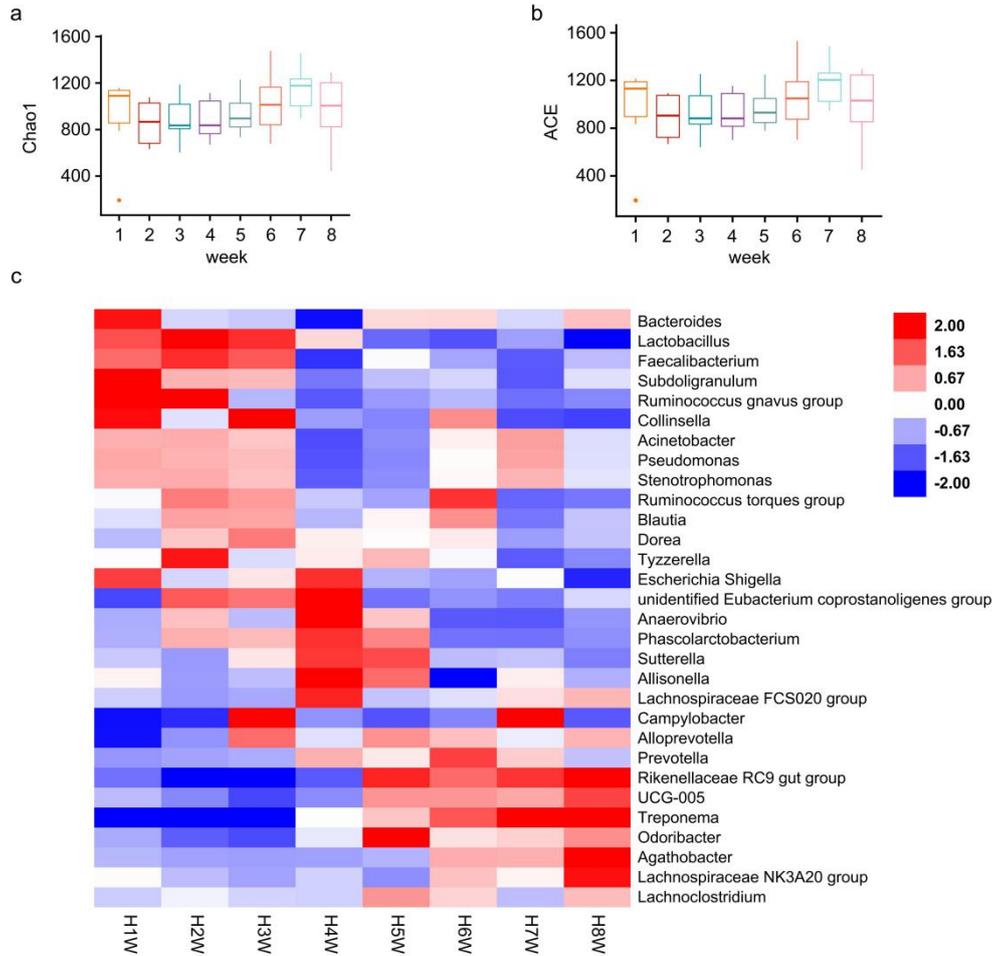
Supplementary Material



Supplementary figure 1. Body parameters after grouping.

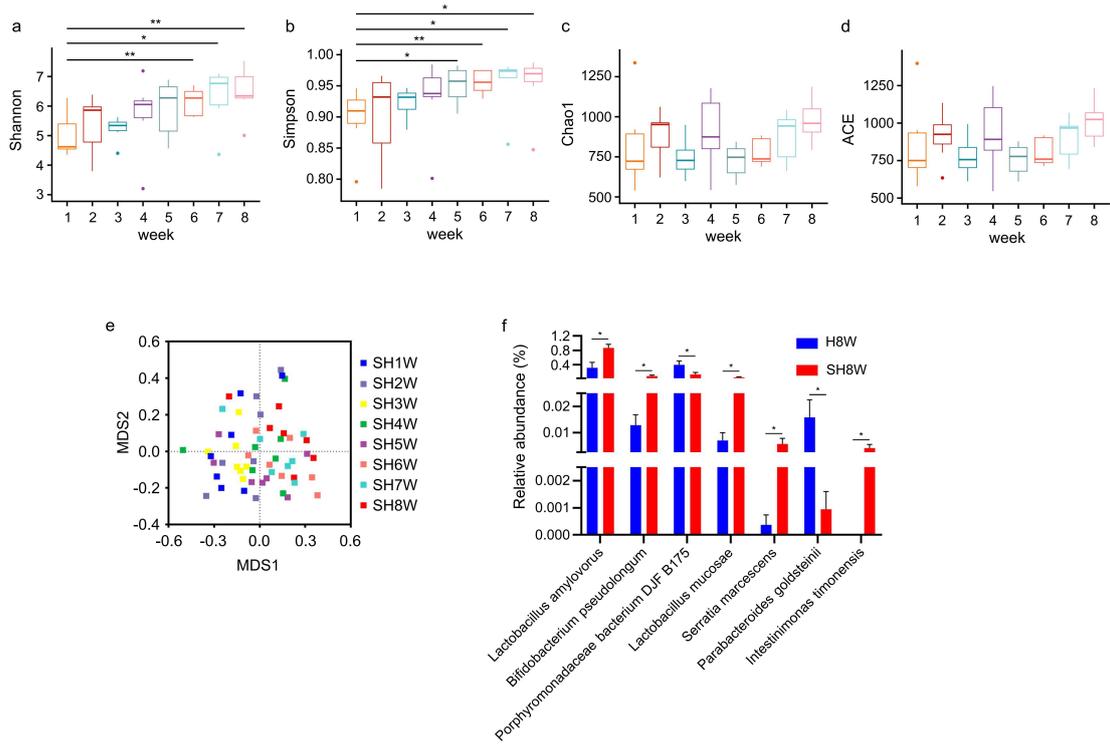
(a) Bodyweight gain of the healthy (n=6) and sub-healthy group (n=7) after grouping. (b-c) After grouping, body height and hip height (b), as well as chest circumference, abdominal circumference and cannon circumference (c) of calves in healthy group and sub-healthy group, were expressed

respectively. Data are shown as mean \pm SEM. *** $p < 0.001$. NS, not statistically significant.



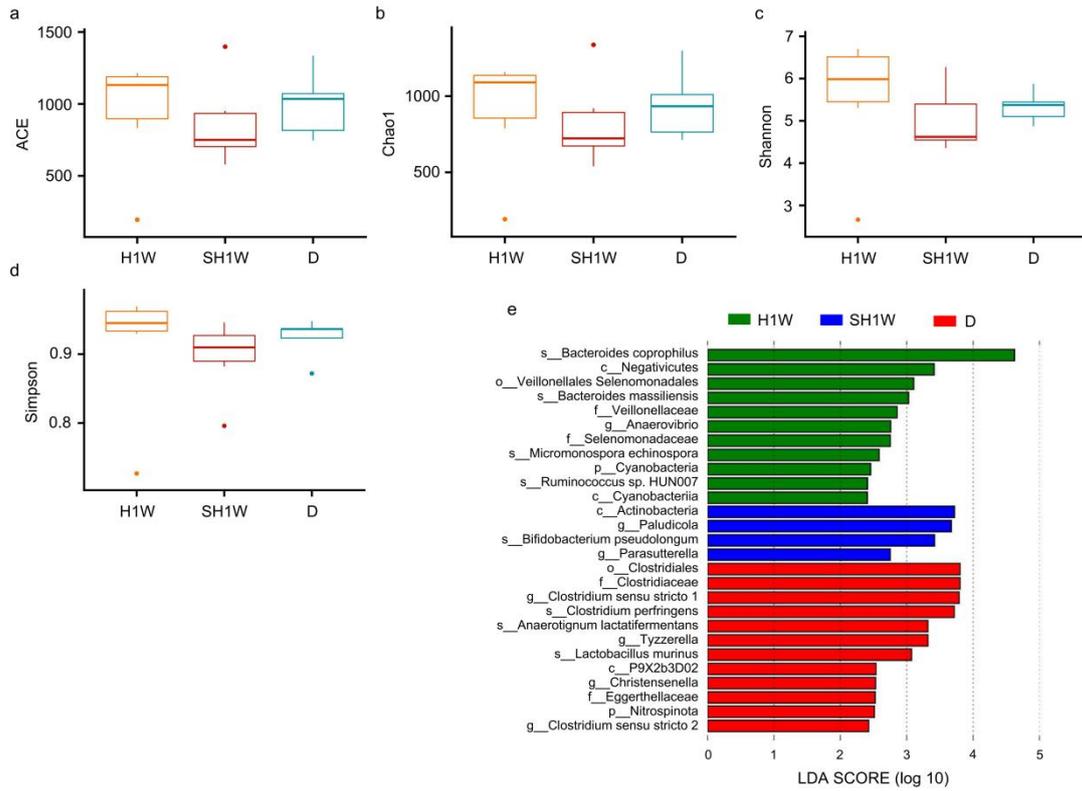
Supplementary figure 2. Intestinal microbiota of healthy calves changes with weekly age.

(a-b) The alpha diversity boxplot of the health group (n=6) was measured using Chao1 and ACE diversity indexes. (c) The relative abundance of bacteria at the genus level in the H group was clustered and represented in a heatmap. Data are shown as mean \pm SEM.



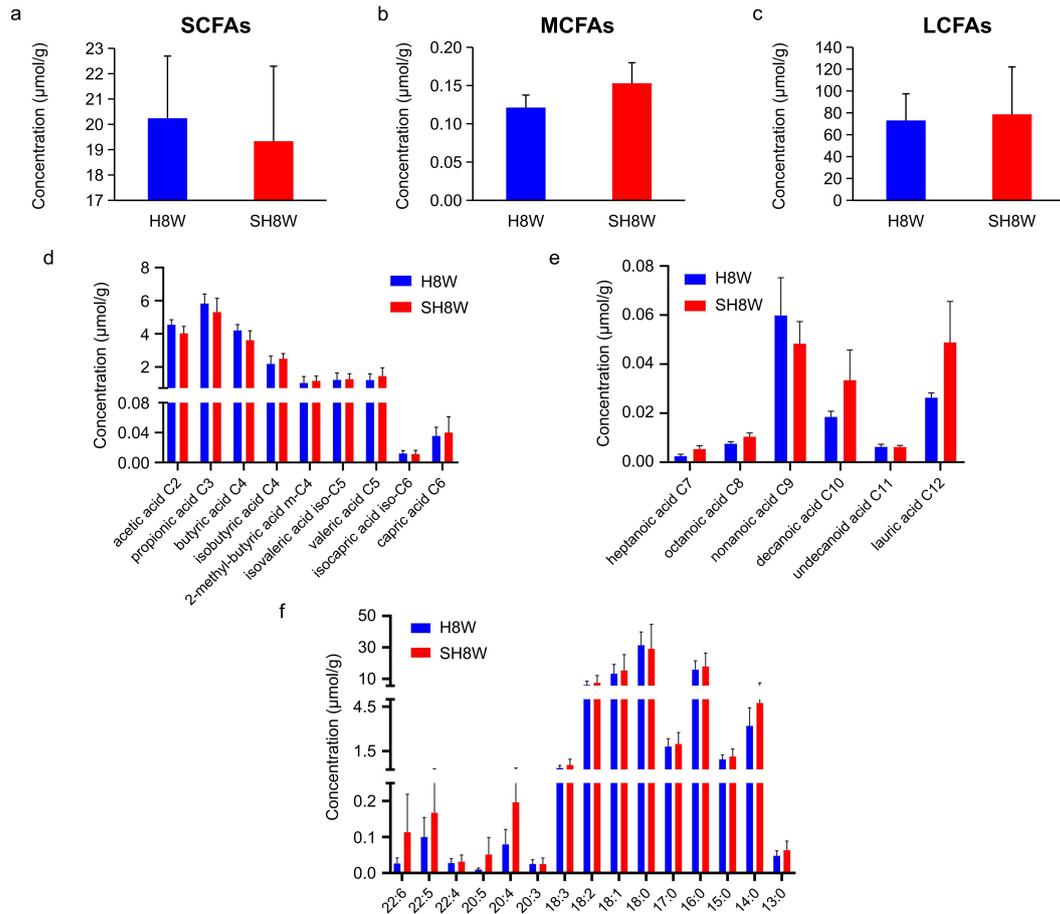
Supplementary figure 3. Comparative analysis of microbe dynamics in the sub-healthy group.

(a-d) The alpha diversity boxplots of the sub-health group (n=7) were measured using Shannon, Simpson, Chao1 and ACE diversity indexes. (e) NMDS of bacterial 16S rRNA gene sequence data from faecal samples of sub-healthy group calves over time was shown. (f) Histogram shows the strains with significant difference between H (n=6) and SH (n=7) group at week 8. Data are shown as mean \pm SEM. *p<0.05, **p<0.01



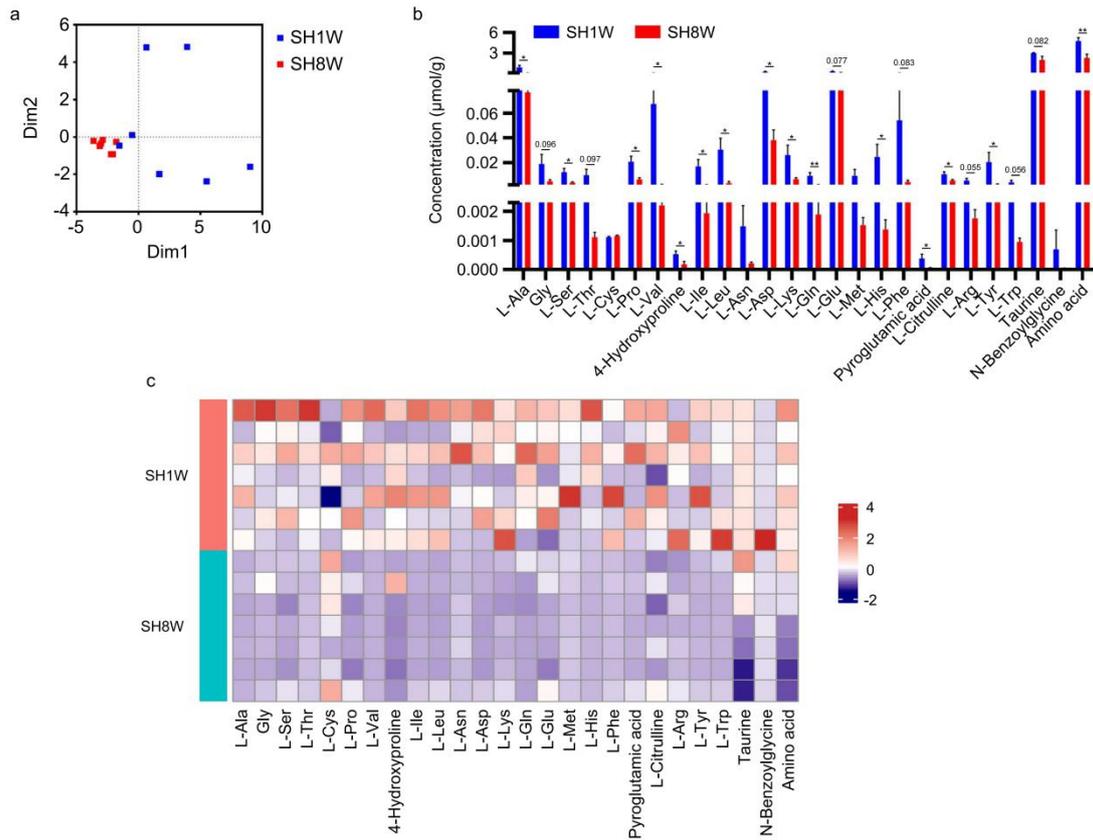
Supplementary figure 4. Comparisons of the intestinal flora among groups H, SH, and D at week 1.

(a-d) The alpha diversity boxplots among H, SH, and D groups (n=6, 7, and 5) were measured using Shannon, Simpson, Chao1 and ACE diversity indexes. (e) The histogram shows the LDA scores computed for OTUs differentially abundant among three groups (\log_{10} LDA > 2.4).



Supplementary figure 5. Difference in faecal fatty acid concentration of 8 week-old calves.

(a-c) Bar plot depicting the total fatty acid concentration of faecal samples at SCFAs (a), MCFAs (b) and LCFAs (c) levels between H (n=6) and SH (n=6) group at week 8. (d-f) Detailed concentrations of fatty acids of different lengths in feces between H and SH groups. Data are shown as mean ± SEM.



Supplementary figure 6. Amino acid concentration decreased with the growth and development of calves.

(a) PCA of amino acid concentration analysis data from faecal samples of SH group (n=7) at weeks 1 and 8 was shown. (b) Bar plot depicting the concentration of different amino acids of sub-healthy calves faecal samples between week 1 and 8. (c) Detailed concentrations of amino acids of SH group at week 1 and 8 were clustered and represented in a heatmap. Data are shown as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$.