

# Effects of medium-chain triglyceride supplementation alone or in combination with $\beta$ -hydroxy- $\beta$ -methylbutyric acid in lactation feed on sow performance and piglet gut microbiota composition

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**ABSTRACT.** To mitigate stress associated with early weaning, modification of sow feeding may indirectly improve the intestinal microbial community in suckling piglets. This study aimed to determine the effects of medium-chain triglycerides (MCT) supplementation, alone or in combination with  $\beta$ -hydroxy- $\beta$ -methylbutyric acid (HMB), on parturition performance, lipid metabolism, and milk composition in sows, as well as on growth performance and intestinal microbiota in piglets, from day 110 of gestation until day 14 postpartum. Thirty multiparous sows (Large White  $\times$  Landrace) in late gestation (parity 2–3) were assigned to three treatment groups: control (basal diet), MCT (basal diet supplemented with 14.3 g MCT), and MCT+HMB (basal diet supplemented with 14.3 g MCT and 2 g HMB). Compared with the control group, the MCT and MCT+HMB groups had significantly shorter labour durations, lower serum interleukin-6 and C-reactive protein concentrations, higher milk fat and protein contents in colostrum, and protein in mature milk, also a higher average daily gain in suckling piglets ( $P < 0.05$ ). Compared with the MCT group, supplementation with MCT+HMB resulted in a higher number of live-born piglets, increased milk protein and fat contents in mature milk, and elevated levels of medium-chain, long-chain, and n-3 polyunsaturated fatty acids in colostrum ( $P < 0.05$ ). Both treatment groups showed a lower relative abundance of harmful bacteria (*Bradyrhizobium* and *Shigella* spp.) and a higher relative abundance of beneficial bacteria (*Bacteroides anomalis* and *Enterococcus faecalis*). These findings suggest that the concurrent supplementation of MCT and HMB is more beneficial for sow productivity and piglet growth performance compared to MCT supplementation alone.

## Introduction

Farrowing and lactation are crucial stages in sow breeding and management. However, many farms have issues with prolonged parturition and insufficient energy reserves in sows, which negatively impact farrowing performance and the growth of nursing piglets (Feyera et al., 2018). Dietary supple-

mentation has been shown to be an effective method to improve sow production performance. Nutrient supplementation before parturition and during lactation can improve delivery performance and lactation capacity of sows, thereby improving piglet growth and favourably influencing the intestinal microbiota of piglets (Oliveira et al., 2020). In suckling piglets, intestinal flora plays a key role in nutrient

metabolism, intestinal development, and immune function (Bauer et al., 2006). Therefore, early stimulation of the intestinal microbiota during the suckling period represents a potential approach to reduce stress associated with early weaning. Interestingly, the composition of the intestinal microbiota and growth performance of suckling piglets can be indirectly improved through maternal nutrition (Zhao et al., 2022). This strategy is safer than direct nutritional alterations in piglet diets, and minimizes the negative effects of human intervention. Consequently, investigating the effects of maternal dietary interventions on the intestinal microbiota of piglets is of considerable importance.

In recent years, medium-chain triglycerides (MCT) have gained popularity owing to their roles in rapid energy supply and regulation of lipid metabolism (Nimbkar et al., 2022). Supplementing sows with medium-chain fatty acids has been shown to affect milk fat composition. In particular, the inclusion of MCT in sow diets during the first seven days after parturition increases the concentration of medium-chain fatty acids (MCFA) in milk (Flummer and Theil, 2012). Jin et al. (2017) added 7.75 g/kg MCT to a sow feed from day 85 of gestation until the end of lactation, resulting in a 37% reduction in farrowing duration, as well as increased colostrum fat and protein content and higher weaning weight of piglets. Similarly, Chen et al. (2019) reported that MCT supplementation in gestating sows increased the proportion of beneficial bacterial genera in the colon of suckling piglets. These results indicate that MCT supplementation can improve both farrowing performance and piglet growth.  $\beta$ -hydroxy- $\beta$ -methylbutyric acid (HMB), an intermediate metabolite of leucine, has been shown to regulate lipid metabolism and reduce chronic inflammation (Morrison and Preston, 2016). Nissen et al. (1994) supplemented sow diets with 2 g HMB per day from 3–4 days before parturition till the end of lactation, which increased colostrum fat content by 41% and piglet weaning weight by 7%. In addition, improvements in lipid metabolism and gut microbiota composition have been demonstrated in a mouse model of diet-induced obesity following HMB supplementation (Duan et al., 2019). In pigs, dietary HMB supplementation during lactation has been associated with increased milk yield and fat content (Flummer and Theil, 2012).

In summary, many studies have confirmed that supplementation with MCT or HMB alone can positively affect pig production. Therefore, we hypothesised that combined supplementation with MCT and HMB in sows may produce synergistic

effects and provide greater benefits than MCT alone. Accordingly, this study was designed to determine the effects of MCT supplementation alone or in combination with HMB and MCT from day 110 of gestation to day 14 of lactation. Supplementation efficiency was assessed based on sow lactation performance, lipid metabolism, and milk composition, as well as piglet growth performance and intestinal microbiota.

## Material and methods

All experimental procedures were approved by the Science and Technology Ethics Committee of Heilongjiang Bayi Agricultural University (Ethics code: DWKJXY2022034) and conducted in accordance with its guidelines. The experiment was carried out at Tonghe Damuren Pig Feeding Co., Ltd. (Harbin, China). The MCT used in the study was prepared as encapsulated solid particles, with a total fatty acid content of 66–72% and an MCT content of  $\geq 70\%$  (Qingdao Yuekang Biotechnology Co., Ltd., Qingdao, China). The encapsulating materials included glucose syrup, casein, potassium citrate, and silica. The HMB was prepared in the form of solid particles, with HMB-Ca as the active component and a total content of  $\geq 99\%$  (Shanxi Fest Biotechnology Co., Ltd., Shaanxi, China).

## Animals and experimental design

Thirty multiparous Large White  $\times$  Landrace sows (2<sup>nd</sup>–3<sup>rd</sup> parity) in late gestation, with backfat thickness ranging from 19 to 27 mm, were assigned to three groups: CON (control), MCT (14.3 g/day medium-chain triglycerides), and MCT+HMB (14.3 g MCT + 2 g HMB). Group allocation was performed to ensure a balanced distribution of backfat thickness. Treatments were administered with the standard diet once daily at 10:00, from day 110 of gestation to day 13 of lactation. The farrowing house maintained weekly disinfection procedures, with *ad libitum* water access and daily manure removal.

The MCT preparation (containing  $\geq 70\%$  active MCT) provided  $\geq 10.0$  g/day of actual medium-chain triglycerides at the 14.3 g/day dosage. The HMB-Ca product (containing  $\geq 99\%$  active ingredient) provided  $\geq 1.98$  g/day of actual HMB at the 2 g/day dosage. Supplements were top-dressed and delivered with the 10:00 meal to ensure complete intake. During gestation, sows were fed 4.5 kg/day of a complete diet, provided as three meals of 1.5 kg (05:00, 10:00, and 16:00). From day 110 of gestation, feeding was adjusted to a restricted lactation regimen. Feed was withheld during parturition and resumed *ad libitum* postpartum. In the MCT+HMB group, this corre-

sponded to 3.18 g MCT product/kg diet (0.318%) and 0.44 g HMB product/kg diet (0.044%).

Neonatal management included umbilical cord processing within 1 h after birth, colostrum administration, and placement in heated incubators with mineral bedding.

Reproductive performance parameters were recorded, including farrowing duration, litter interval, number of live-born, stillborn, and mummified piglets, as well as overall piglet health status. Backfat thickness was measured using an ultrasonic backfat meter before parturition and on day 21 of lactation to assess body reserve mobilisation. Six piglets per litter were randomly selected for weight monitoring on days 14 and 21. Creep feed was introduced on day 7 and weaning was performed on day 21.

The basal diet was formulated to meet or exceed the nutrient requirements for late-gestation and lactating sows, according to NRC (2012) recommendations. The ingredient composition and calculated nutrient contents are shown in Table 1. Feed samples were analysed using AOAC Official Methods of Analysis (AOAC International, 2019) to verify key nutrient contents, including crude protein and minerals. Feed hygiene and contaminant control complied with the national hygienic standard (GB 13078-2017).

**Table 1.** The composition of basal feed, served for sows during 96 days of gestation to rebreeding

Item	Value
Ingredient composition, %	
corn	64.03
flour	6.00
43% soybean meal	16.50
fish meal (piglet)	1.00
expanded soybean	4.50
bran	3.50
soybean oil	0.80
calcium hydrogen phosphate type iii	1.00
stone powder	1.50
calcium hydrogen phosphate type iii	0.48
lysine hydrochloride (98%)	0.20
threonine	0.10
valine	0.10
premix <sup>1</sup>	0.85
sodium chloride	0.45
total	100
Nutrient concentrations, %	
net energy of breeding pigs, mcal/kg	2.45
crude protein	15.82
lysine	0.83
calcium	0.94
total phosphorus	0.66
available phosphorus	0.41

<sup>1</sup> provided per kg of the diet: IU: vit. A 70 000, vit. D 2 085, vit. E 100; mg: vit. K<sub>3</sub> 4.52, vit. B<sub>1</sub> 1.81, pyridoxine 4.76, riboflavin 5.98, niacin 29.78, D-pantothenic acid 35.79, folic acid 3.58, biotin 0.59, 60%-choline chloride 0.4; µg: vit. B<sub>12</sub> 30

## Collection of colostrum and mature milk

Colostrum was collected on the day of parturition, whereas mature milk on day 14 after parturition. Milk samples were obtained from the anterior, middle, and posterior mammary glands of each sow, pooled into 10 ml Eppendorf tubes and stored at -20 °C until further analysis.

## Colostrum and mature milk composition analysis

Milk composition, including fat, lactose, protein, and non-fat solids, was determined using a milk composition analyser (LACTOSCAN, Milkotronic Ltd., Bulgaria).

## Blood sample collection

Blood samples were collected within 3 h after the end of parturition and on day 14 of lactation. Samples were obtained from the marginal ear vein of sows into 10 ml EP tubes. After clotting for 30 min, blood samples were centrifuged at 4 000 rpm for 15 min rpm (iCen24, Hangzhou Allsheng Instruments Co., Ltd., Hangzhou, China) to separate the serum. The resulting serum was aliquoted into 1.5 ml EP tubes and stored at -20 °C until further analysis.

## Analysis of serum biochemical indicators

Serum samples were analysed for inflammatory and lipid metabolism parameters. Inflammatory markers included interleukin-6 (IL-6), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), and C-reactive protein (CRP). Lipid metabolism parameters comprised total cholesterol (TC), triglycerides (TG), non-esterified fatty acid (NEFA), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). All parameters were determined using commercially available kits (Harbin Scientia Biotechnology Co., Ltd., Harbin, China), according to the manufacturers' instructions.

## Data recording and sample collection

Eighteen healthy piglets were randomly selected from each group. Faecal samples were collected at 7, 14, and 21 days of age into Eppendorf tubes. Samples were obtained using rectal swabs and immediately stored in liquid nitrogen.

## Microbiome profiling

Total DNA was extracted from faecal samples (faeces and swabs) using a combined chemical lysis and phenol-chloroform purification protocol. Briefly, mechanical and enzymatic lysis was performed using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany), followed by organic phase separation and DNA precipitation.

The hyper variable V3–V4 regions of bacterial 16S rRNA genes were amplified by PCR using the primers 341F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGGTWTCTAAT-3'). Sequencing libraries were prepared using the TruSeq DNA PCR-Free Kit (Illumina, San Diego, CA) and quantified by Qubit fluorometry (Thermo Fisher Scientific). Sequencing was performed on an Illumina NovaSeq 6000 platform (PE250), with library preparation and sequencing carried out by Shanghai Payson Biotechnology Co. (Shanghai, China).

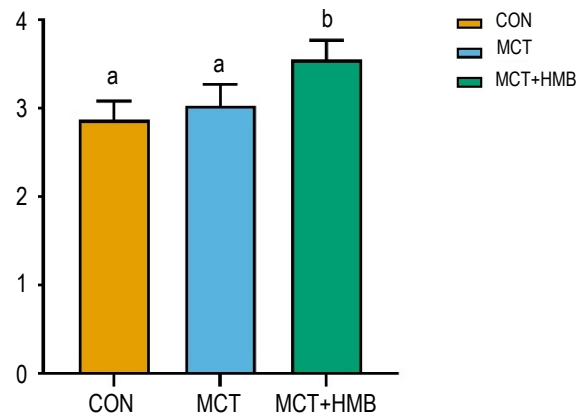
## Results

### Sow delivery performance

As shown in Table 2, no significant differences were observed between the groups in total litter size, numbers of healthy, mummified, or stillborn piglets ( $P > 0.05$ ). However, farrowing duration and the interval between piglets were significantly shorter in the MCT and MCT+HMB groups compared with the control group ( $P < 0.05$ ).

### Backfat loss

As shown in Figure 1, backfat loss in sows was significantly greater in the MCT+HMB group than in the control and MCT groups ( $P < 0.05$ ). No significant difference in backfat loss was observed between the MCT and control groups ( $P > 0.05$ ).



**Figure 1.** The backfat loss of sows

CON – control, MCT – medium-chain triglycerides, HMB –  $\beta$ -hydroxy- $\beta$ -methylbutyric acid; values are presented as means  $\pm$  SEM; ab – superscripts indicate significant differences among groups at  $P < 0.05$ , whereas the same superscript letters indicate no significant difference  $P > 0.05$

no significant differences were found among the three groups in IL-6, CRP, and TNF- $\alpha$  concentrations ( $P > 0.05$ ).

### Serum lipid metabolic factors

On the day of parturition, sows in the MCT group had a higher concentration of NEFA ( $P < 0.05$ ), lower of LDL-C ( $P < 0.05$ ), a tendency towards increased HDL-C concentrations ( $P = 0.07$ ; Table 3) compared with the control group (Table 2). In the MCT+HMB group, TC and LDL-C

**Table 2.** Effects of medium-chain triglycerides supplementation (MCT) or MCT and  $\beta$ -hydroxy- $\beta$ -methylbutyric acid (HMB) on farrowing performance in sows

Item	Treatments			SEM	P-value		
	CON	MCT	MCT+HMB		CON vs MCT	CON vs MCT+HMB	MCT vs MCT+HMB
Total born	13.30	13.10	13.80	0.49	0.83	0.23	0.16
Born alive	12.60	12.70	13.30	0.33	0.41	<0.05	0.20
Stillborn	0.70	0.40	0.50	0.30	0.35	0.59	0.69
Health born	11.90	11.60	11.90	0.44	0.62	0.91	0.70
Weak born	0.70	1.10	1.40	0.25	0.25	<0.05	0.39
Mummified	0.30	0.20	0.40	0.17	0.63	0.89	0.54
Delivery duration, h	3.87	3.45	3.50	0.09	<0.05	<0.05	0.30
Farrowing interval, h	0.29	0.26	0.25	0.01	<0.05	<0.05	0.14

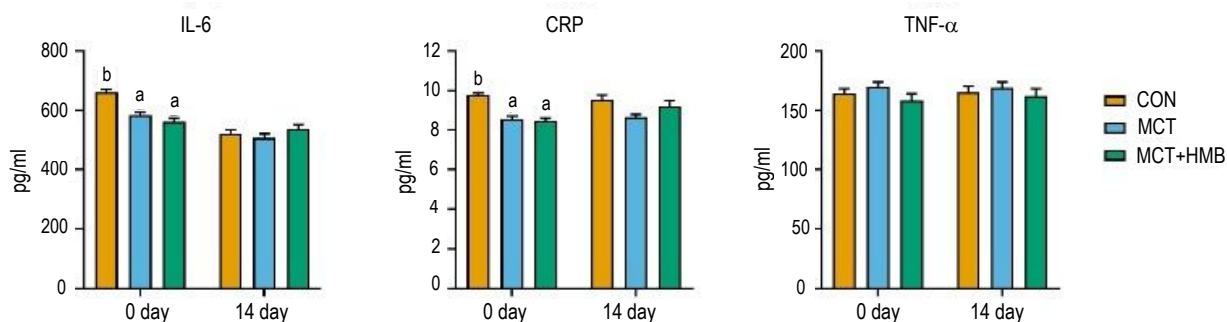
CON – control diet; SEM – standard error of the mean; values are presented as means  $\pm$  SEM;  $P < 0.05$  was considered statistically significant

### Analysis of serum biochemical indicators

**Blood inflammatory factors in postpartum sows.** The concentrations of IL-6, CRP, and TNF- $\alpha$  in sows after parturition are presented in Figure 2. On the day of parturition, the MCT and MCT+HMB groups had lower serum IL-6 and CRP levels compared to the control group ( $P < 0.05$ ), while there was no significant difference in TNF- $\alpha$  concentrations between the groups ( $P > 0.05$ ). On day 14 postpartum,

concentrations were elevated ( $P < 0.05$ ), while NEFA concentrations were reduced ( $P < 0.05$ ) than in the control group. Compared with the MCT group, the MCT+HMB animals showed lower TC concentrations ( $P < 0.05$ ), and a tendency towards reduced TG concentrations ( $P = 0.054$ ).

On day 14 after parturition, TC and LDL-C concentrations were significantly lower in both the MCT and MCT+HMB groups than in controls ( $P < 0.05$ ).



**Figure 2.** The interleukin-6 (IL-6), C-reactive protein (CRP), and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) concentrations of the sows after parturition CON – control, MCT – medium-chain triglycerides, HMB –  $\beta$ -hydroxy- $\beta$ -methylbutyric acid; values are presented as means  $\pm$  SEM; ab – superscripts indicate significant differences among groups at  $P < 0.05$ , whereas the same superscript letters indicate no significant difference  $P > 0.05$

**Table 3.** Effect of medium-chain triglycerides supplementation (MCT) or MCT and  $\beta$ -hydroxy- $\beta$ -methylbutyric acid (HMB) on serum lipid metabolic factors in sows, mmol/l

Item	Treatments			SEM	P-value		
	CON	MCT	MCT+HMB		CON vs MCT	CON vs MCT+HMB	MCT vs MCT+HMB
0 day							
TC	1.30	1.34	1.14	0.03	1.00	<0.05	<0.05
TG	1.26	1.28	1.15	0.03	1.00	0.12	0.054
NEFA	0.59	0.73	0.74	0.03	<0.05	<0.05	1.00
HDL-C	0.71	0.90	0.87	0.05	0.07	0.16	1.00
LDL-C	0.62	0.50	0.45	0.02	<0.05	<0.05	0.57
14 days							
TC	1.87	1.39	1.10	0.08	<0.05	<0.05	0.055
TG	1.23	1.24	1.24	0.01	0.99	0.29	1.00
NEFA	0.63	0.62	0.70	0.04	1.00	0.85	0.55
HDL-C	1.06	1.13	1.21	0.02	0.19	<0.05	<0.05
LDL-C	0.81	0.53	0.55	0.02	<0.05	<0.05	1.00

CON – control diet; TC – total cholesterol, TG – triglycerides, NEFA – non-esterified fatty acids, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol; SEM – standard error of the mean; values are presented as means  $\pm$  SEM;  $P < 0.05$  was considered statistically significant

Additionally, HDL-C levels were higher in the MCT+HMB group compared with both the control and MCT groups ( $P < 0.05$ ). A decreasing trend in TC concentration was also observed in the MCT+HMB group compared with the MCT group ( $P = 0.055$ ).

### Colostrum and ordinary milk composition analysis

Colostrum fat and protein contents were higher in the MCT and MCT+HMB groups than in the control group ( $P < 0.05$ ). Moreover, colostrum protein content was higher in the MCT+HMB group than in the MCT group ( $P < 0.05$ ; Table 4). No significant differences were observed between the three groups in lactose or solids-not-fat content in colostrum ( $P > 0.05$ ).

Protein content in mature milk was higher in the MCT and MCT+HMB groups than in the control group ( $P < 0.05$ ; Table 4). Fat content was also higher in the MCT+HMB group compared with the

control ( $P < 0.05$ ), whereas no significant difference was observed between the MCT and control animals ( $P > 0.05$ ). No significant differences were found between the MCT and MCT+HMB groups ( $P > 0.05$ ), nor in lactose and non-fat solid contents among the three experimental treatments ( $P > 0.05$ ).

### Growth performance of piglets

No significant differences were observed in the average birth weight of piglets between the treatment groups ( $P > 0.05$ ; Table 5). At 14 and 21 days of age, piglets in the MCT and MCT+HMB groups had higher body weights than those in the control ( $P < 0.05$ ), with no differences between the two supplemented groups. Average daily gain (ADG) was higher in the MCT and MCT+HMB groups over the periods 1–14, 14–21, and 1–21 days compared to the control group ( $P < 0.05$ ), with no differences recorded between the experimental groups.

**Table 4.** Effect of medium-chain triglycerides supplementation (MCT) or MCT and  $\beta$ -hydroxy- $\beta$ -methylbutyric acid (HMB) on the composition of colostrum and milk in sows

Item	Treatments			SEM	P-value		
	CON	MCT	MCT+HMB		CON vs MCT	CON vs MCT+HMB	MCT vs MCT+HMB
Colostrum							
fat, %	3.74	4.16	4.49	0.11	<0.05	<0.05	0.15
lactose, %	9.13	9.72	9.28	0.27	0.34	1.00	0.91
protein, %	9.61	11.4	13.1	0.28	<0.05	<0.05	<0.05
non-fat solid, %	20.3	21.6	20.6	0.61	0.34	1.00	0.92
Ordinary milk							
fat, %	3.42	3.52	3.56	0.03	0.21	<0.05	1.00
lactose, %	3.50	3.54	3.42	0.04	1.00	0.63	0.17
protein, %	3.68	3.78	3.81	0.02	<0.05	<0.05	0.75
non-fat solid, %	7.77	7.87	7.63	0.10	1.00	0.92	0.29

CON – control diet; SEM – standard error of the mean; values are presented as means  $\pm$  SEM;  $P < 0.05$  was considered statistically significant

**Table 5.** Effects of medium-chain triglycerides supplementation (MCT) or MCT and  $\beta$ -hydroxy- $\beta$ -methylbutyric acid (HMB) on piglet growth, kg

Item	Treatments			SEM	P-value		
	CON	MCT	MCT+HMB		CON vs MCT	CON vs MCT+HMB	MCT vs MCT+HMB
Weight on day of age, day							
1	1.45	1.51	1.5	0.11	0.69	0.74	0.94
14	4.11	4.51	4.66	0.13	<0.05	<0.05	0.39
21	5.64	6.34	6.61	0.19	<0.05	<0.05	0.31
Average weight gains, days							
1–14	0.19	0.21	0.23	0.01	<0.05	<0.05	0.39
14–21	0.22	0.26	0.27	0.01	<0.05	<0.05	0.31
1–21	0.2	0.23	0.24	0.03	<0.05	<0.05	0.35

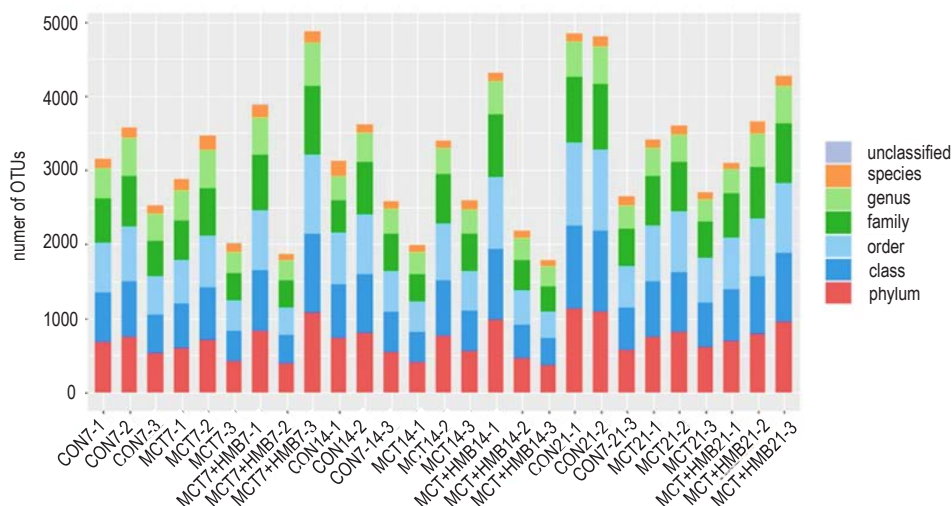
CON – control diet; SEM – standard error of the mean; values are presented as means  $\pm$  SEM;  $P < 0.05$  was considered statistically significant

## Sequence information

After sequencing 27 samples, a total of 21 428 660 valid sequences were obtained. These sequences were rarefied and clustered into operational taxonomic units (OTUs) at a 97% similarity threshold. The results of taxonomic annotation are presented in Figure 3.

## Alpha and beta diversity analysis

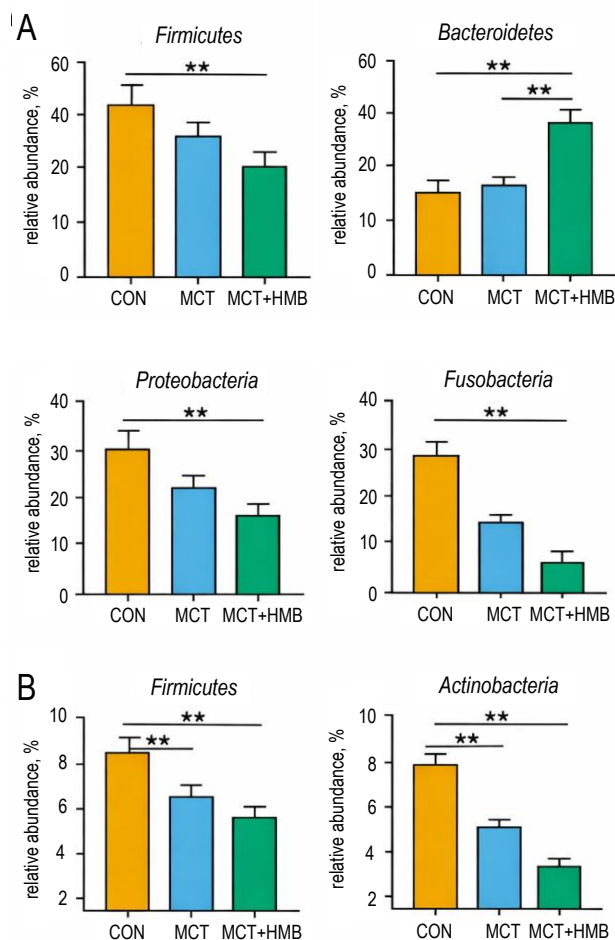
No significant differences were observed in alpha and beta diversity indices of the intestinal microbiota among the treatment and control groups ( $P > 0.05$ ), nor between the treatment groups at days 7, 14, and 21 ( $P > 0.05$ ; Supplementary Figure 1).



**Figure 3.** Sequencing sequence annotation results of piglet gut microbes  
CON – control, MCT – medium-chain triglycerides, HMB –  $\beta$ -hydroxy- $\beta$ -methylbutyric acid

## Differences among dominant phyla genera

The composition of intestinal microbial phyla in piglets across treatment groups and time points, including taxa with relative abundances above 1% was analysed (Supplementary Figure 2; Figure 4). The relative abundance of intestinal microbial phyla at 7 days of age was not significantly different between the control and MCT groups ( $P > 0.05$ ). However, the relative abundance of *Firmicutes*, significantly decreased in the MCT+HMB supplemented animals ( $P < 0.05$ ). At 14 days of age, no significant differences in relative abundance were observed between the treatment groups ( $P > 0.05$ ). At 21 days of age, the relative abundance of the phyla *Firmicutes* ( $P < 0.05$ ) significantly decreased in the MCT and MCT+HMB groups compared to the control group (Supplementary Figure 2; Figure 4).



**Figure 4.** Differences in the relative abundance of dominant bacterial phyla in piglets at 7 and 21 days of age. (A) Relative abundance of dominant bacterial phyla in 7-day-old piglets. (B) Relative abundance of dominant bacterial phyla in 21-day-old piglets

CON – control, MCT – medium-chain triglycerides, HMB –  $\beta$ -hydroxy- $\beta$ -methylbutyric acid; values are presented as means  $\pm$  SEM; \*\* –  $P < 0.01$

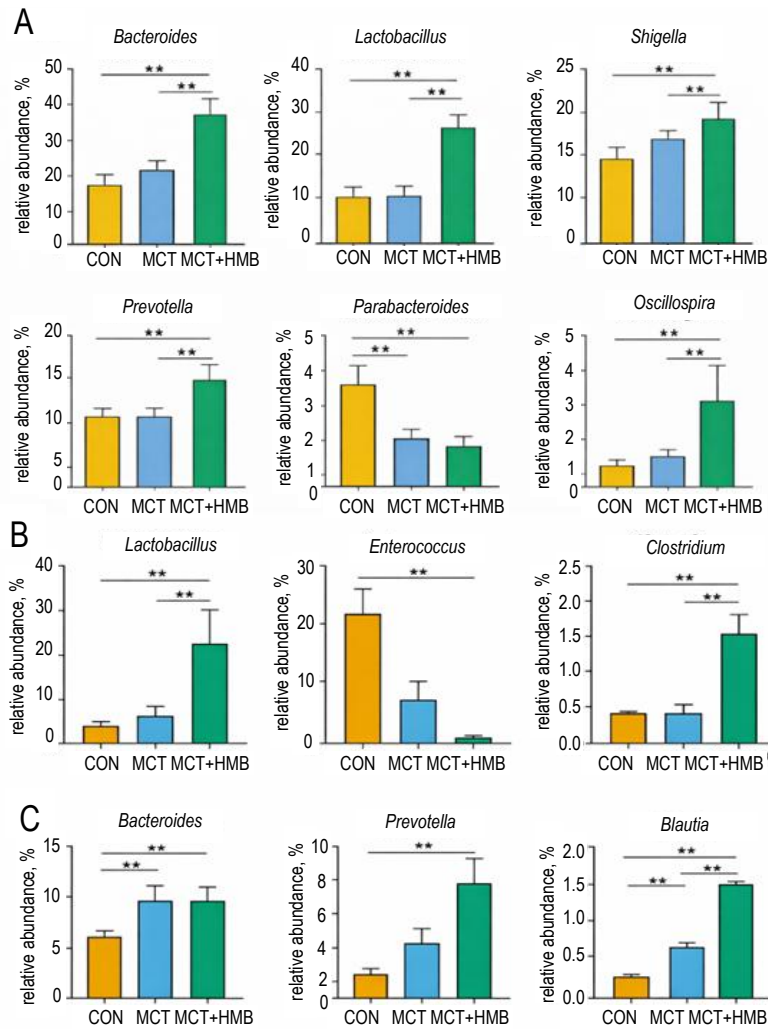
## Differences in dominant genera

Intestinal microbial communities at the genus level (relative abundance  $> 1\%$ ) were evaluated across treatment groups and time points (Supplementary Figure 3), with differences in relative abundance presented in Figure 5. At 7 days of age, the relative abundance of *Anaplasma* spp., *Lactobacillus* spp., *Prevotella* spp., and *Treponema* spp. in the MCT + HMB group significantly increased ( $P < 0.05$ ) compared to the control group, whereas that of *Shigella* spp. and *Paraplasma* spp. significantly decreased ( $P < 0.05$ ). In contrast, the relative abundance of *Paraplasma* spp. in the MCT group was significantly reduced ( $P < 0.05$ ) compared to the control group. Additionally, when comparing the MCT+HMB group to the MCT group, the relative abundance of *Prevotella* spp. and *Treponema* spp. ( $P < 0.05$ ) in the former significantly increased, while that of *Shigella* spp. significantly decreased ( $P < 0.05$ ). At 14 days of age, the MCT+HMB group showed higher relative abundance of *Lactobacillus* spp. and lower abundance of *Clostridium* spp. compared to the control treatment ( $P < 0.05$ ). The MCT+HMB group had higher abundance of *Lactobacillus* spp. and *Clostridium* spp. compared to the MCT group. At 21 days of age, significant differences in the relative abundance of several taxa were observed among treatment groups, with *Prevotella* showing a significantly higher relative abundance in the MCT+HMB group than in the control group ( $P < 0.05$ ).

## Determination of time-associated marker genera and analysis of variance

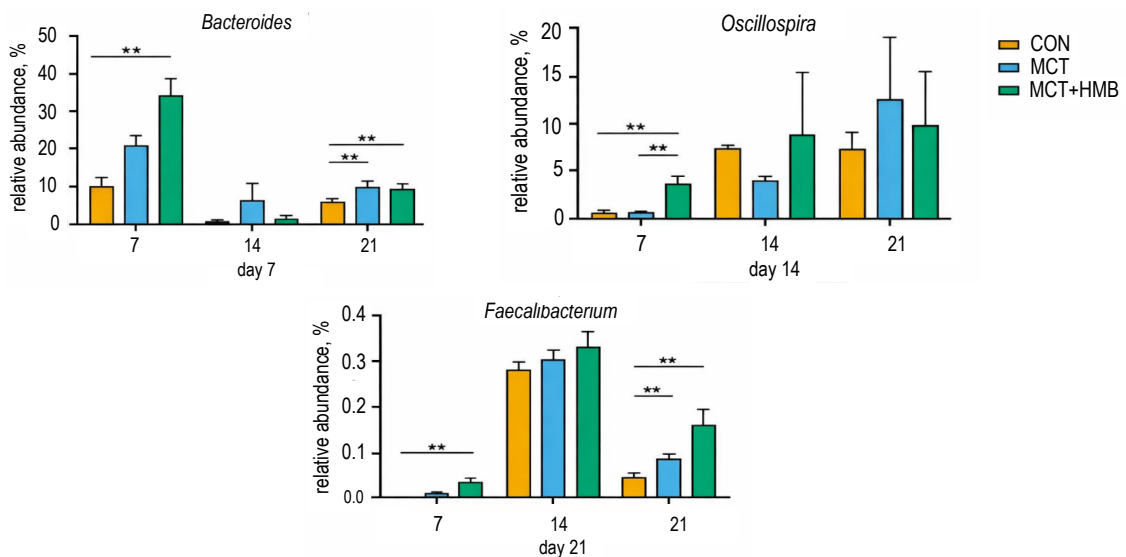
The variable importance analysis of intestinal microbial genera using the Random Forest machine-learning algorithm identified thirteen genera and ranked them based on their significance (Supplementary Figure 4).

ANOVA was performed on all time-associated genera within each treatment group at the three time points to identify taxa showing intergroup differences (Figure 6). The relative abundance of *Anabaena* spp. decreased with age. At 7 days of age, their relative abundance in the MCT+HMB treatment group was significantly higher than in the control group ( $P < 0.05$ ), while at 21 days of age both the MCT and MCT+HMB groups contained more of these bacteria than the control ( $P < 0.05$ ). In contrast, *Treponema* spp. numbers increased with age, as at 7 days of age, their relative abundance in the MCT+HMB treatment was significantly higher compared to the control ( $P < 0.05$ ). At 7 days of age, the relative abundance of



**Figure 5.** Differences in the relative abundance of dominant bacterial genera in piglets at 7, 14, and 21 days of age. (A) Relative abundance of dominant bacterial genera in 7-day-old piglets. (B) Relative abundance of dominant bacterial genera in 14-day-old piglets. (C) Relative abundance of dominant bacterial genera in 21-day-old piglets

CON – control, MCT – medium-chain triglycerides, HMB –  $\beta$ -hydroxy- $\beta$ -methylbutyric acid; values are presented as means  $\pm$  SEM; \* –  $P < 0.05$ ; \*\* –  $P < 0.01$



**Figure 6.** Differences in the relative abundance of time-series marker genera in piglets at 7, 14, and 21 days of age

CON – control, MCT – medium-chain triglycerides, HMB –  $\beta$ -hydroxy- $\beta$ -methylbutyric acid; values are presented as means  $\pm$  SEM; \*\* – significant difference between groups at  $P < 0.01$

*E. faecalis* in the MCT+HMB group was significantly higher compared to controls animals ( $P < 0.05$ ), and at 21 days of age both the MCT and MCT+HMB groups also contained relatively more of these bacteria than the control group ( $P < 0.05$ ).

ANOVA was performed on all time-associated genera within each treatment group at the three time points to identify taxa showing intergroup differences (Figure 6). The relative abundance of *Anabaena* spp. decreased with age. At 7 days of age, their relative abundance in the MCT+HMB treatment group was significantly higher than in the control group ( $P < 0.05$ ), while at 21 days of age both the MCT and MCT+HMB groups contained more of these bacteria than the control ( $P < 0.05$ ). In contrast, *Treponema* spp. numbers increased with age, as at 7 days of age, their relative abundance in the MCT+HMB treatment was significantly higher compared to the control ( $P < 0.05$ ). At 7 days of age, the relative abundance of *E. faecalis* in the MCT+HMB group was significantly higher compared to controls animals ( $P < 0.05$ ), and at 21 days of age both the MCT and MCT+HMB groups also contained relatively more of these bacteria than the control group ( $P < 0.05$ ).

## Discussion

### Effects of MCT and MCT+HMB on sow parturition performance

Careful feeding and management are important during sow parturition due to the high energy demand associated with the farrowing process. Owing to its metabolic properties, MCT can be used as a rapidly available energy source for sows. Previous research showed that supplementation with 20 g/day MCT (50% active ingredient) from 5 days before parturition and 40 g of MCT on the day of parturition reduced labour duration by 29–51% in sows of parity 1–6 (Li et al., 2015). In the present study, supplementation with 14.3 g/day MCT from gestational day 110 to parturition reduced labour time by 10% and litter interval by 6.8%. Co-supplementation with MCT and HMB produced similar reductions, decreasing labour duration by 10% and litter interval by 10%. Compared with previous reports, the magnitude of the reduction was smaller, which may be attributed to the relatively low-parity sows (parity 2–3) used in this experiment, as these animals already displayed shorter farrowing time in the control group, limiting the potential for further improvement through supplementation.

Due to the high number of foetuses carried by modern high-yielding sows and the limited uterine space, some foetuses may suffer from developmental constraints in terms of space and nutrition (Strange et al., 2013). This in turn can lead to incomplete development and the birth of weaker piglets. In this study, the MCT+HMB group showed a significant increase in the number of viable piglets, which may indicate improved farrowing assistance and the successful delivery of weaker piglets that would otherwise have had a lower chance of survival in the other groups. Prolonged parturition can damage the reproductive tract, leading to inflammation and impaired farrowing performance as well as delayed postpartum recovery (Schoos et al., 2020). In this study, both experimental groups showed a reduction in labour time and lower postpartum serum concentrations of IL-6 and CRP, suggesting that a shorter parturition process may reduce reproductive tract damage. However, no significant differences were observed in serum TNF- $\alpha$  between the three groups, which may be explained by the greater sensitivity of TNF- $\alpha$  to pathogen-induced rather than physiological inflammation (Liu et al., 2023).

### Effect of MCT and MCT+HMB on sow lipid metabolism, milk composition, and piglet growth performance

The energy balance and lipid metabolism rate in lactating sows are closely related to backfat loss and milk quality (Hansen et al., 2012). Lipid metabolism is commonly evaluated through serum indicators, including TC, TG, NEFA, LDL-C, and HDL-C (Qi et al., 2020). Previous studies demonstrated that replacing soybean oil with a diet containing 15.7% MCT during late gestation increased serum free fatty acid concentrations in sows (Newcomb et al., 1991).

In the current study, both the MCT and MCT+HMB groups showed increased serum NEFA concentrations and decreased LDL-C concentrations on the day of parturition, as well as reduced TC and LDL-C concentrations on day 14 postpartum, indicating an effect of both supplements on lipid metabolism in sows. On the day of parturition, serum TC was lower in the MCT+HMB group than in the MCT group, whereas HDL-C was higher in the MCT+HMB group compared to the MCT group on day 14 postpartum. These differences may be related to distinct mechanisms of lipid metabolism regulation induced by MCT and HMB. MCT has been reported to increase the levels of hormone sensitive

lipase and upstream protein kinase A, thereby promoting lipolysis (Abe, 2022).

Sow body condition is generally assessed by backfat thickness. Excessive backfat may cause lactation disorders and negatively affect the growth of piglets (Papadopoulos et al., 2022). Previous studies have shown that supplementation with 2 g/day HMB during the 2–3 days before parturition increased backfat loss during lactation, with similar effects observed at a daily dose of 5.2 g HMB (Hu et al., 2020). In the present study, MCT supplementation alone exerted no significant effect on backfat loss during lactation; however, co-supplementation with MCT and HMB significantly increased this process. This indicates that HMB could enhance lipolysis and milk fat synthesis at the expense of body fat reserves, while MCT co-supplementation did not suppress this effect. This may be due to the relatively low amount of MCT provided, which was insufficient to compensate for the additional energy demand associated with HMB-mediated regulation of lipid metabolism.

Milk is the only energy source for neonatal piglets, and its quality largely determines early growth performance (Baxter et al., 2023). Milk fat represents a key energy component and plays an important role in regulating piglet growth and intestinal development (You et al., 2023). Previous studies in sows have shown that MCT supplementation (7.75 g/kg from day 85 of gestation to parturition) increased colostrum fat and protein content, while HMB similarly increased fat and protein in both colostrum and mature milk (Newcomb et al., 1991). Here, colostrum fat and protein contents were significantly increased in both the MCT and MCT+HMB groups compared with the control.

MCT regulates sow lipid metabolism, increasing serum NEFA availability, thereby providing additional substrates for milk fat synthesis (Gerlinger et al., 2011). The higher colostrum fat content observed in the MCT+HMB group compared to the MCT group may be attributed to a stronger modulatory effect of HMB on lipid metabolism, resulting in a greater supply of fatty acid precursors for milk fat synthesis. Similarly, colostrum protein content increased significantly in both experimental groups compared to control animals, suggesting that MCT promoted protein synthesis in colostrum. The further increase observed in the MCT+HMB group, compared with the MCT group, could be associated with the known protein synthesis-stimulating effects of HMB.

MCT and HMB also positively affected mature milk production in sows. Previous findings showed that supplementation with 5.2 g/day HMB during

late pregnancy and lactation increased fat percentage and protein content in mature milk (Flummer and Theil, 2012), and adding 4 g/kg MCT for one week antepartum and during lactation could significantly increase fat percentage and dry matter content in mature milk (Zentek et al., 2011). In this study, protein content in mature milk was higher in both the MCT and MCT+HMB groups compared to the control group. Milk fat percentage was also higher in the MCT+HMB group than in the control group, whereas no significant differences were observed between the MCT and control groups. These results indicated that MCT alone, or combined with HMB, exerts similar effects on mature milk protein content, whereas improvements in milk fat percentage appear to be mainly associated with HMB-mediated metabolic regulation rather than MCT supplementation alone.

Newborn piglets store energy mainly in the form of glycogen and a small amount of fat. Glycogen is rapidly utilised after birth, and postnatal energy demands are primarily met by colostrum and mature milk (Jarratt et al., 2023). Therefore, piglet weight gain can also reflect sow lactation performance. Previous studies demonstrated that sow dietary supplementation with 7.75 g/kg MCT during late pregnancy and lactation significantly improved the growth performance of piglets (You et al., 2023). In the current study, both the MCT and MCT+HMB groups had higher average weight and ADG of piglets at 14 and 21 days of age compared with control animals, which was consistent with the results of previous studies. Although large differences in colostrum fatty acid composition were observed between the two supplemented groups, this did not translate into differences in piglet growth performance. This suggests that improved growth performance of piglets may be associated with increased milk yield rather than changes in milk composition alone.

### **Effect of MCT and MCT+HMB on the intestinal microbiota of piglets**

No significant differences were observed in alpha diversity or beta diversity indices of the piglet intestinal microbiota, which may be attributed to the low microbial abundance and the unstable internal environment in pre-weaned piglets. In addition, the dose and combination ratio of the additives may also explain the lack of detectable effects on overall microbial diversity.

The dominant phyla of the intestinal flora in suckling piglets varied over time, indicating a continuous change in the relative abundance of the dominant phyla during lactation, as reported

previously (Li et al., 2018). Although the MCT group showed a reduced relative abundance of specific bacterial taxa at 21 days of age, no significant differences were observed at 7 and 14 days of age. However, diversity analysis revealed subtle structural changes in the intestinal microbiota, with significant differences detected at 21 days of age. The similarity between the treatment groups at 14 days of age may be related to the introduction of creep feeding, which likely influenced microbial composition. At the genus level, the dominant intestinal microorganisms in suckling piglets also varied over time, reflecting ongoing changes in their relative abundance during development (Arrieta et al., 2014). Genera with a relative abundance above 1% at each time point also differed significantly between groups. The highest number of significant differences was observed at 7 days of age, suggesting that the effects on the piglet gut microbiota were most pronounced during early lactation, likely due to changes in colostrum composition. In addition, we hypothesised that the timing of nutrient additions was equally important, and alterations in the feeding schedule could also influence the gut flora; this should be explored in future studies. For example, *Prevotella* is a common member of the intestinal microbiota and may contribute to carbohydrate fermentation and metabolite production related to host metabolism.

Conversely, pathogenic bacteria such as *Shigella* spp. are associated with intestinal disorders in piglets (Monticelli et al., 2018). In the present study, the MCT + HMB treatment significantly increased the relative abundance of beneficial genera such as *Prevotella* and *Treponema*, while reducing the relative abundance of pathogenic bacteria such as *Shigella* spp. compared to the MCT treatment. These findings are consistent with those reported by Chen et al. (2019). Overall, combined supplementation with MCT and HMB produced more pronounced beneficial effects than MCT alone. Early-life establishment of the intestinal microbiota plays an important role in piglet growth by contributing to the maturation of the digestive and mucosal immune systems, supporting intestinal barrier function, and limiting the proliferation of opportunistic pathogens (Nowland et al., 2022). Here, the reduced relative abundance of potentially pathogenic taxa (e.g., *Shigella*) together with the enrichment of commensal taxa in the treatment groups may indicate a more stable intestinal ecosystem. Such a microbial profile is generally considered beneficial for gut health and may allow piglets to allocate more nutrients and energy towards growth rather than responding to intestinal disturbances (Dou et al., 2017; Guevarra et al., 2019).

The composition of the intestinal microbiota undergoes continuous changes and maturation during the lactation stage of piglets. The balance of gut microorganisms during this critical period has a significant impact on subsequent development (Huang et al., 2019). In the present study, a random forest-based Boruta algorithm was used to identify genera with distinct temporal patterns in the gut microbiota of piglets aged 7 to 21 days. *Anaplasma* spp., *Treponema* spp., and *Faecalobacterium* spp. demonstrated intergroup differences and were associated with temporal variation during lactation. Significant differences in all three genera were observed at 7 days of age, which may have been related to changes in colostrum composition. At 14 days of age, these differences were not significant, potentially due to disruption of the gut flora following the introduction of creep feed. However, differences re-emerged at 21 days of age, when piglets had adapted to solid feed. These findings indicate that maternal supplementation with MCT or MCT+HMB can affect the intestinal microbiota of piglets during lactation, increasing the abundance of beneficial genera, with stronger effects observed for combined supplementation.

## Conclusions

From gestational day 110 to postnatal day 14, supplementation with medium-chain triglycerides (MCT) alone or in combination with  $\beta$ -hydroxy- $\beta$ -methylbutyric acid (HMB) improved the delivery performance of sows. The combined MCT+HMB treatment was more effective than MCT alone, as reflected by an increased number of live-born piglets, higher protein content, and elevated levels of medium-chain fatty acids, long-chain fatty acids, and n-3 polyunsaturated fatty acids in colostrum, as well as increased fat content in mature milk. Both MCT alone or combined MCT plus HMB supplementation improved piglet growth performance to a similar extent. Changes in milk composition were associated with a reduced abundance of harmful bacteria such as *Firmicutes*, *Aspergillus*, and *Shigella* and an increased abundance of beneficial bacteria, including *Anaplasma*, *Treponema*, and *E. faecalis* in the intestinal tract of piglets. These effects were more prominent in the MCT+HMB group.

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## Conflict of interest

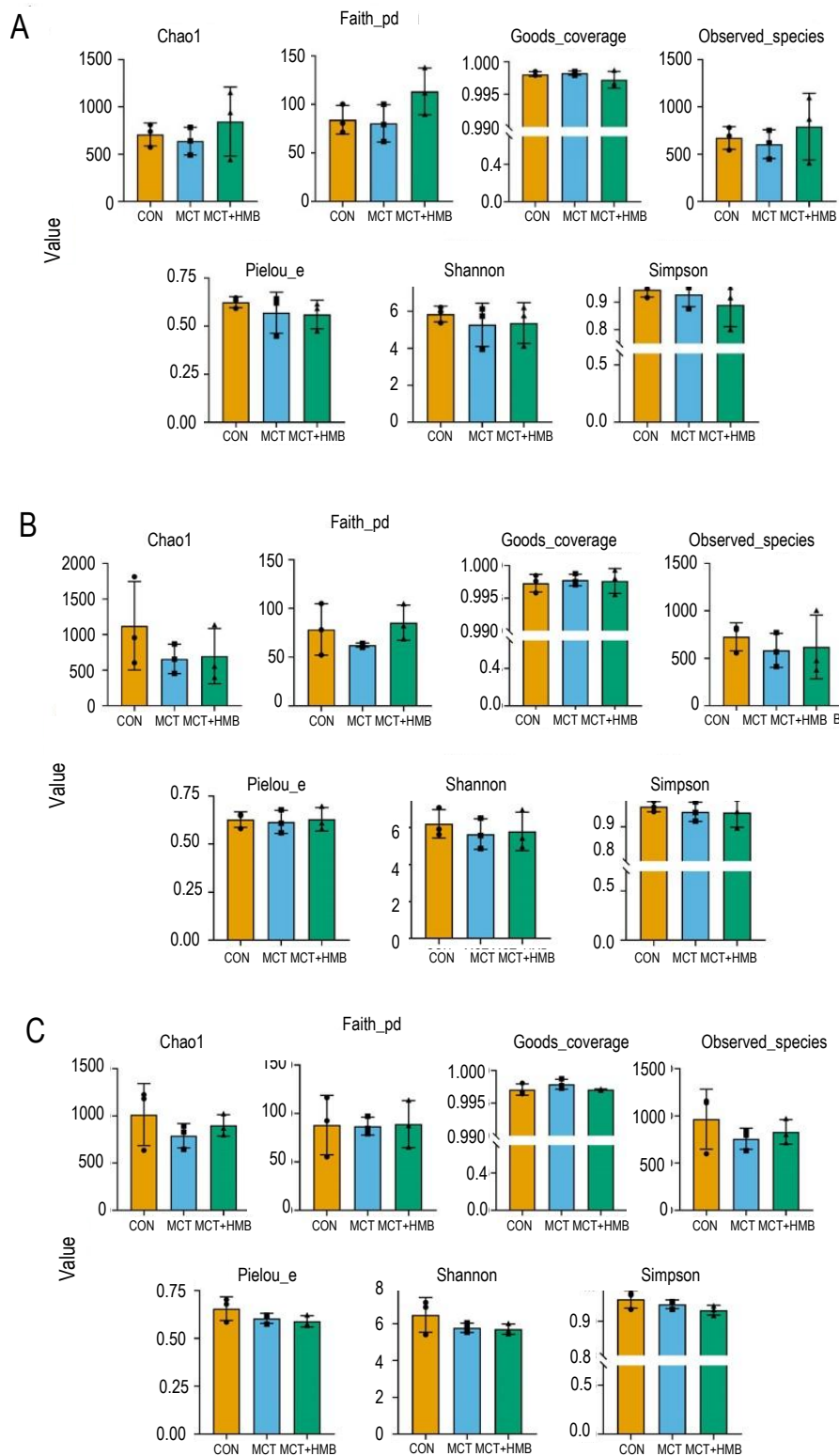
The Authors declare that there is no conflict of interest. Author Xiaoming Liu is employed by Da Mu Ren Animal Husbandry (Heilongjiang) Group Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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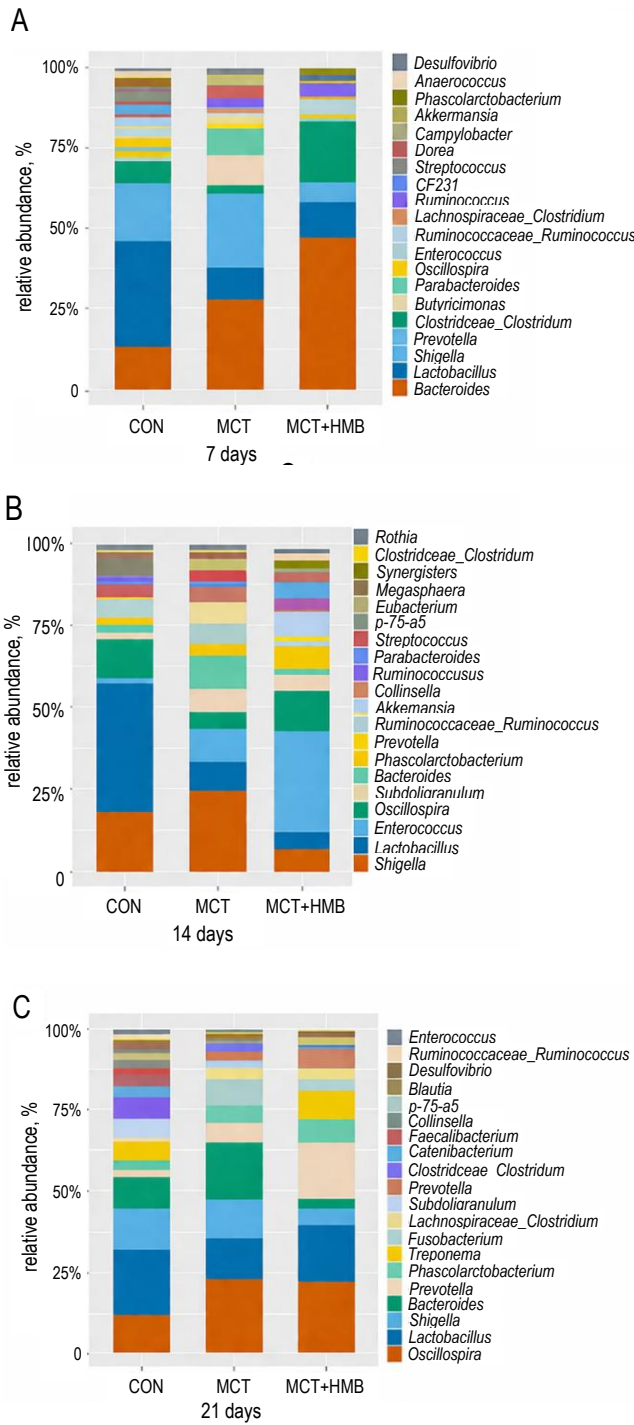
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## Supplementary figures



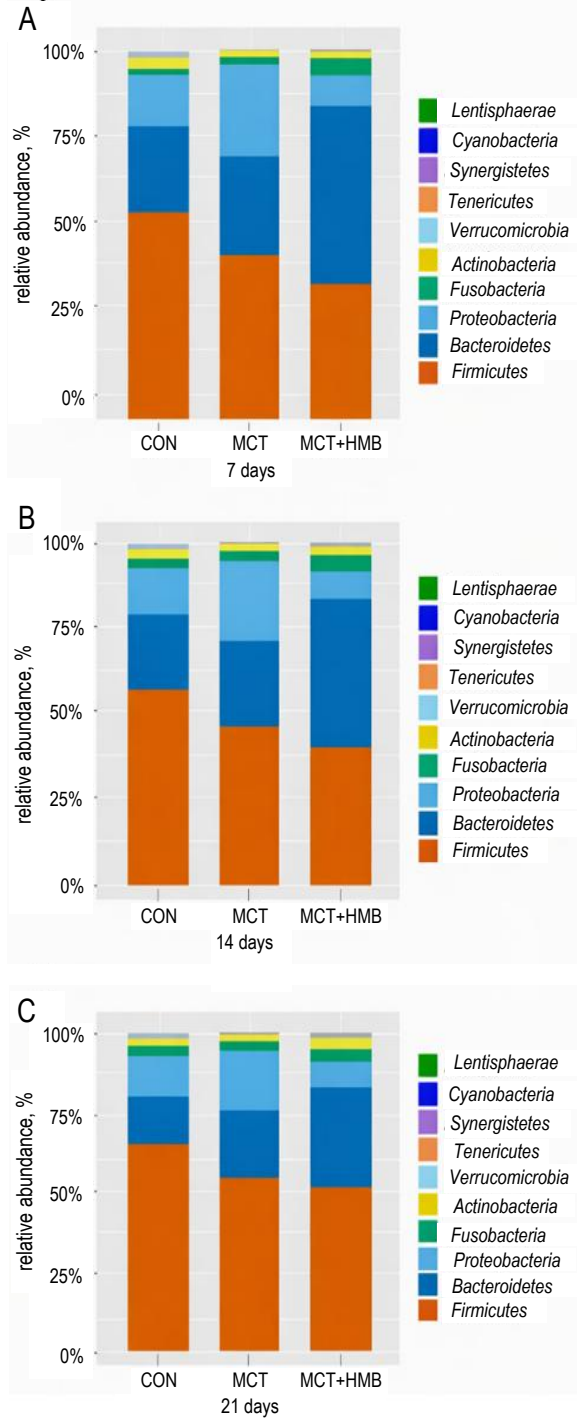
**Supplementary figure 1.** Alpha diversity indices of the gut microbiota in piglets at 7, 14, and 21 days of age. (A) Alpha diversity indices of the gut microbiota in 7-day-old piglets. (B) Alpha diversity indices of the gut microbiota in 14-day-old piglets. (C) Alpha diversity indices of the gut microbiota in 21-day-old piglets

CON – control, MCT – medium-chain triglycerides, HMB –  $\beta$ -hydroxy- $\beta$ -methylbutyric acid; Chao1 – Chao1 richness index, Faith\_pd – Faith's phylogenetic diversity, Goods\_coverage – Good's coverage, Observed\_species – observed species richness, Pielou\_e – Pielou's evenness index, Shannon – Shannon diversity index, Simpson – Simpson diversity index; values are presented as means  $\pm$  SEM



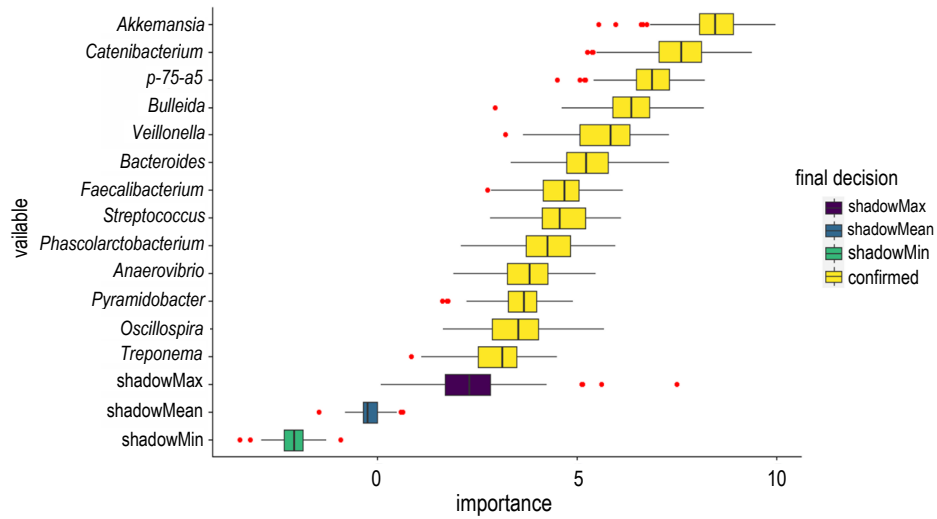
**Supplementary figure 2.** Stacked bar plot of the relative abundance of dominant bacterial genera in piglets at 7, 14, and 21 days of age. (A) Relative abundance of dominant bacterial genera in 7-day-old piglets. (B) Relative abundance of dominant bacterial genera in 14-day-old piglets. (C) Relative abundance of dominant bacterial genera in 21-day-old piglets

CON – control, MCT – medium-chain triglycerides, HMB –  $\beta$ -hydroxy- $\beta$ -methylbutyric acid



**Supplementary figure 3.** Stacked bar plot of the relative abundance of dominant bacterial phyla in piglets at 7, 14, and 21 days of age. (A) Relative abundance of dominant bacterial phyla in 7-day-old piglets. (B) Relative abundance of dominant bacterial phyla in 14-day-old piglets. (C) Relative abundance of dominant bacterial phyla in 21-day-old piglets

CON – control, MCT – medium-chain triglycerides, HMB –  $\beta$ -hydroxy- $\beta$ -methylbutyric acid



**Supplementary figure 4.** Importance ranking of temporal marker genera identified by Boruta analysis. Bacterial genus names are shown in italics. Confirmed indicates genera confirmed as important variables by the Boruta algorithm. shadowMax, shadowMean, and shadowMin indicate the maximum, mean, and minimum importance of shadow features, respectively. Importance represents the variable importance score.