

纯种柯乐猪与巴×柯杂交猪肠道菌群结构的研究

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摘 要: 本试验对纯种柯乐猪与巴×柯杂交猪肠道菌群结构特性及差异性进行了研究,旨在从猪肠道微生物的角度揭示纯种柯乐猪耐粗饲特性及巴×柯杂交猪对青绿饲料的适应能力,为后期微生物添加剂的设计、柯乐猪杂交利用及标准化养殖提供依据。纯种柯乐猪和巴×柯杂交猪在同等条件下养殖,达屠宰体重后(100 kg左右),随机各选3头屠宰,取十二指肠、空肠、回肠、结肠内容物,进行高通量测序和生物学信息分析。结果显示:纯种柯乐猪与巴×柯杂交猪各肠段Alpha多样性指数差异不显著($P>0.05$);Beta多样性分析显示,2个品种猪大部分样品交叉聚集到一起;肠道菌群结构分析显示,纯种柯乐猪和巴×柯杂交猪小肠阶段以厚壁菌门(Firmicutes)、变形菌门(Proteobacteria)和放线菌门(Actinobacteria)为优势菌门,到了结肠阶段则是以拟杆菌门(Bacteroidetes)和厚壁菌门为优势菌门;纯种柯乐猪与巴×柯杂交猪结肠中有大量的纤维分解菌属,巴×柯杂交猪结肠中月形单胞菌属(*Selenomonas*)和脱硫弧菌属(*Desulfovibrio*)的相对丰度显著或极显著高于纯种柯乐猪($P<0.05$ 或 $P<0.01$),而消化球菌属(*Peptococcus*)、聚乙酸菌属(*Acetitomaculum*)、*Leeia*的相对丰度则显著低于纯种柯乐猪($P<0.05$);LEfSe分析后发现纯种柯乐猪结肠中富集着大量的产短链脂肪酸菌,其中纤维降解菌有聚乙酸菌属、瘤胃球菌科的不可培养瘤胃细菌4C0d_17(uncultured_rumen_bacterium_4C0d_17)、*Leeia*、拟杆菌纲、拟杆菌目,与纤维降解菌起协同作用的菌群有考拉杆菌属(*Phascolarctobacterium*)、考拉杆菌属的uncultured_Veillonellaceae_bacterium;巴×柯杂交猪结肠中富集的纤维降解菌有月形单胞菌属、柔嫩梭菌(*Clostridium leptum*)。上述结果表明,在整个肠道菌群结构上,纯种柯乐猪和巴×柯杂交猪的优势菌在类别上非常接近,体现了环境相同效应,纯种柯乐猪和巴×柯杂交猪的肠道菌群结构相对稳定且相似度高;在属水平上,纯种柯乐猪和巴×柯杂交猪的结肠中纤维降解菌表现出差异,纯种柯乐猪结肠内纤维降解菌的比例较高,说明引入外源血对肠道菌群结构有一定的影响,巴×柯杂交猪对粗纤维的消化能力较弱于柯乐猪。

关键词: 纯种柯乐猪;巴×柯杂交猪;肠道菌群;高通量测序;生物信息学分析

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柯乐猪是我国优良地方猪种之一,属于乌金猪系列的一个品种,被列入《中国畜禽遗传资源

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志·猪志》,毛色有黑、鼠灰、“六白”、棕色 4 种,主产于贵州省毕节市赫章县,具有抗病力强、适应性强、耐粗饲、肉质鲜美、口感糯而细腻等特点,是“宣威火腿”的原材料,目前以高端猪肉原料进行市场开发^[1-2]。但柯乐猪是脂肪型猪,存在瘦肉率低等问题,相关研究表明,柯乐猪达到最佳屠宰期(体重 100 kg 左右)时,瘦肉率仅为(43.48±4.45)%^[3-4],阻碍了市场的开发。巴克夏猪是英国古老的培育猪种之一,中国华南猪对巴克夏猪有过种源贡献,巴克夏猪以黑毛为主,具有“一黑六白”特点,嘴筒、四肢、尾尖毛色为白色,其余部分是黑色^[5],巴克夏猪生长速度快、瘦肉率高^[6]。为了提高柯乐猪的瘦肉率,保持以黑毛为主的特点,采用巴克夏猪为父本、柯乐猪为母本进行杂交生产 F1 代猪(以下简称巴×柯杂交猪)。

相关研究表明,不同品种的猪肠道菌群结构是具有差异的^[7],不同猪品种进行杂交能发挥最大的杂交优势,提高生长性能、肉质、繁殖力等^[8]。但不同品种的猪进行杂交后,其肠道菌群结构是否具有差异的研究相关报道较少。因此,本试验研究纯种柯乐猪与巴×柯杂交猪肠道菌群结构特性及差异性,旨在揭示柯乐猪耐粗饲特性及巴×柯杂交猪对粗纤维的消化能力,为后期微生物添加剂的设计及巴×柯杂交猪标准化养殖打下基础。

1 材料与方法

1.1 试验方法

动物试验地点在贵州省毕节市赫章县优农谷生态有限公司申家沟柯乐猪繁育场。选取柯乐猪、巴克夏猪公猪各 1 头,柯乐猪母猪 16 头,随机分成 2 组: I 组,1 头柯乐猪♂+8 头柯乐猪♀; II 组,1 头巴克夏猪♂+8 头柯乐猪♀。分别对 2 组中的公猪进行采精,用所采集的精液对组内母猪进行人工授精,母猪配种成功后,饲养管理、营养水平、免疫程序均相同。仔猪断奶后,各随机选育 9 头健康、体重相近的纯种柯乐猪和巴×柯杂交猪进行饲养,共 18 头,所有饲养条件相同。

1.2 样品采集

8 月龄时纯种柯乐猪和巴×柯杂交猪各随机选体重(体重达 100 kg 左右)相近的 3 头健康猪(3 头纯种柯乐猪体重分别为 108、106、105 kg; 3 头巴×柯杂交猪体重分别为 106、105、102 kg)进行屠宰,共屠宰 6 头,屠宰后每头猪采取十二指肠、空

肠、回肠、结肠 4 个肠段内容物于灭菌的 10 mL 离心管中,放于-78.5 °C 干冰中保存,送至微基因科技(上海)有限公司(<http://www.tinygene.com>)进行 MiSeq 文库制备(QIAamp DNA Stool Mini Kit 试剂盒进行基因组 DNA 抽提,PCR 扩增,AxyPrepDNA 凝胶回收试剂盒回收,FTC-3000™ real-time PCR 仪进行实时荧光定量 PCR),而后进行 Illumina MiSeq 2×300 bp 高通量测序与生物信息学分析[操作分类单元(OTU)聚类分析、Alpha 多样性分析、Beta 多样性分析等]。

1.3 统计分析

对试验所得数据用 Excel 2016 进行初步处理后,Alpha 多样性相关数据采用 SAS 9.2 软件进行显著性分析,高通量测序数据采用 Metastats (<http://metastats.cbcb.umd.edu/>) 在不同分类学水平上进行显著性分析, $P < 0.05$ 表示差异显著, $P < 0.01$ 表示差异极显著。

2 结果与分析

2.1 OTU 信息统计与聚类分析

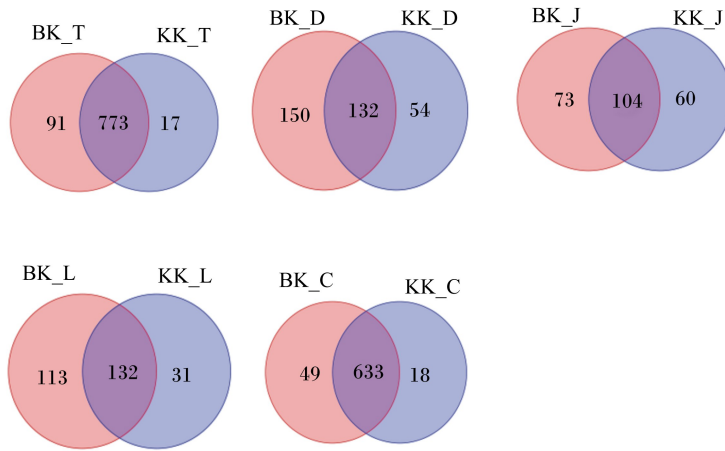
所有样品的优化序列为 862 381 条,纯种柯乐猪有 422 847 条优化序列,巴×柯杂交猪有 439 534 条优化序列;根据 97% 相似性水平,对所有序列进行 OTU 聚类分析,韦恩图(图 1)显示,整个肠道,纯种柯乐猪和巴×柯杂交猪共有 OTU 为 773 个,纯种柯乐猪特有 OTU 为 17 个,巴×柯杂交猪特有 OTU 为 91 个;十二指肠段,纯种柯乐猪和巴×柯杂交猪共有 OTU 为 132 个,纯种柯乐猪特有 OTU 为 54 个,巴×柯杂交猪特有 OTU 为 150 个;空肠段,纯种柯乐猪和巴×柯杂交猪共有 OTU 为 104 个,纯种柯乐猪特有 OTU 为 60 个,巴×柯杂交猪特有 OTU 为 73 个;回肠段,纯种柯乐猪和巴×柯杂交猪共有 OTU 为 132 个,纯种柯乐猪特有 OTU 为 31 个,巴×柯杂交猪特有 OTU 为 113 个;OTU 最多的肠段是结肠,纯种柯乐猪和巴×柯杂交猪共有 OTU 为 633 个,纯种柯乐猪特有 OTU 为 18 个,巴×柯杂交猪特有 OTU 为 49 个。由稀释曲线(图 2)可知,随着序列数增加,曲线趋向于平坦,其覆盖指数均为 0.999~1.000,说明测序数据量合理,可以进行数据分析。

2.2 Alpha 多样性分析

如表 1 所示,从 Chao 指数和 ACE 指数看,纯种柯乐猪与巴×柯杂交猪之间差异不显著($P >$

0.05),说明这 2 个品种猪之间肠道微生物群落丰富度基本一致;从 Shannon 指数和 Simpson 指数看,纯种柯乐猪和巴×柯杂交猪之间差异不显著 ($P>0.05$),说明这 2 个品种猪之间肠道微生物群

落多样性基本一致;随着肠道自上而下,纯种柯乐猪和巴×柯杂交猪肠道微生物群落丰富度及多样性逐渐增加。



BK: 巴×柯杂交猪;KK: 纯种柯乐猪;T: 全肠道;D: 十二指肠;J: 空肠;L: 回肠;C: 结肠。BK: Berkshire×Kele hybrid pigs; KK: pure-bred Kele pigs; T: total intestine; D: duodenum; J: jejunum; L: ileum; C: colon. The same as below.

图 1 巴×柯杂交猪与纯种柯乐猪各肠段 OTU 韦恩图

Fig.1 OTU Venn diagram of each intestinal segment of Berkshire×Kele hybrid pigs and pure-bred Kele pigs

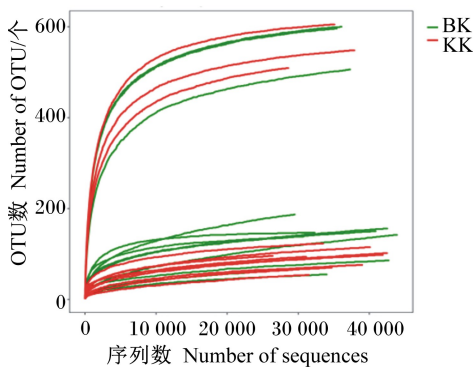


图 2 稀释曲线

Fig.2 Dilution curve

2.3 Beta 多样性分析

通过主成分分析 (PCA, 图 3) 和主坐标分析 (PCoA, 图 4) 比较纯种柯乐猪与巴×柯杂交猪肠道菌群结构相似性,可以发现,2 个品种猪的大部分样品交叉聚集到一起,这说明纯种柯乐猪与巴×柯杂交猪肠道微生物存在一定的相似性,肠道菌群结构组成基本相似。

2.4 菌群结构分析

2.4.1 十二指肠菌群结构分析

由图 5 可知,巴×柯杂交猪十二指肠中的主要

菌门为无壁菌门 (Tenericutes) (34.11%)、厚壁菌门 (Firmicutes) (26.78%)、变形菌门 (Proteobacteria) (28.16%)、拟杆菌门 (Bacteroidetes) (5.02%);纯种柯乐猪十二指肠中的主要菌门为厚壁菌门 (49.04%)、无壁菌门 (40.53%)、变形菌门 (9.32%)。由表 2 可知,巴×柯杂交猪十二指肠中拟杆菌门的相对丰度显著高于纯种柯乐猪 ($P<0.05$)。由图 6 可知,纯种柯乐猪和巴×柯杂交猪十二指肠中的主要菌属为支原体属 (*Mycoplasma*)、乳杆菌属 (*Lactobacillus*)、曲杆菌属 (*Curvibacter*)。由表 3 可知,巴×柯杂交猪十二指肠中克雷伯氏菌属 (*Klebsiella*) 的相对丰度极显著高于纯种柯乐猪 ($P<0.01$),拟杆菌属 (*Bacteroides*) 的相对丰度显著高于纯种柯乐猪 ($P<0.05$),*Mogibacterium*、假丁酸弧菌属 (*Pseudoscardovia*) 的相对丰度显著低于纯种柯乐猪 ($P<0.05$)。

2.4.2 空肠菌群结构分析

由图 5 可知,巴×柯杂交猪空肠中的主要菌门为厚壁菌门 (89.40%)、变形菌门 (9.81%)、放线菌门 (Actinobacteria) (0.48%);纯种柯乐猪空肠中的主要菌门为厚壁菌门 (98.21%)、放线菌门 (0.92%)、无壁菌门 (0.84%)。由图 6 可知,乳杆

菌属(>35.25%) 在纯种柯乐猪与巴×柯杂交猪空肠中均占有绝对优势。由表 3 可知,巴×柯杂交猪空肠中拟杆菌属、厌氧弧菌属(*Anaerovibrio*)、瘤胃

球菌属(*Ruminococcus*) 的相对丰度显著高于纯种柯乐猪($P<0.05$)。

表 1 纯种柯乐猪和巴×柯杂交猪肠道微生物群落 Alpha 多样性分析

Table 1 Alpha diversity analysis of intestinal microbiota of pure-bred Kele pigs and Berkshire×Kele hybrid pigs

项目 Items	肠道 Intestine	巴×柯杂交猪 BK	纯种柯乐猪 KK	均值标准误 SEM	P 值 P-value
Chao 指数 Chao index	十二指肠 Duodenum	191.628 4	109.587 9	31.110 7	0.136
	空肠 Jejunum	150.666 7	151.319 4	37.671 1	0.991
	回肠 Ileum	195.459 6	126.923 1	37.671 1	0.175
	结肠 Colon	607.932 2	581.047 0	20.372 2	0.404
ACE 指数 ACE index	十二指肠 Duodenum	198.887 3	115.532 6	35.818 0	0.175
	空肠 Jejunum	180.133 3	171.958 4	28.055 7	0.847
	回肠 Ileum	259.827 9	153.345 9	28.055 7	0.199
	结肠 Colon	597.780 1	577.088 7	24.024 7	0.575
Shannon 指数 Shannon index	十二指肠 Duodenum	2.327 8	1.356 3	0.259 8	0.057
	空肠 Jejunum	1.409 4	1.484 9	0.601 3	0.933
	回肠 Ileum	2.356 2	2.093 4	0.601 3	0.602
	结肠 Colon	4.932 3	4.920 4	0.571 5	0.965
Simpson 指数 Simpson index	十二指肠 Duodenum	0.250 6	0.445 7	0.095 6	0.222
	空肠 Jejunum	0.481 2	0.431 7	0.202 6	0.871
	回肠 Ileum	0.153 8	0.220 9	0.202 6	0.494
	结肠 Colon	0.019 9	0.019 9	0.004 9	0.999

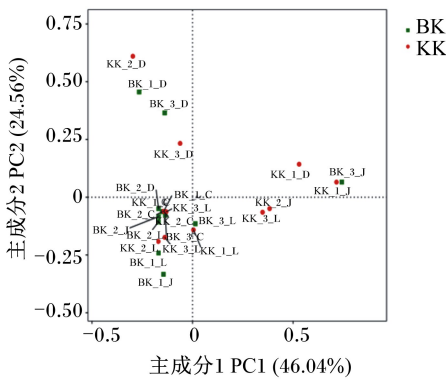


图 3 主成分分析
Fig.3 PCA

2.4.3 回肠菌群结构分析

由图 5 可知,巴×柯杂交猪回肠中的主要菌门为厚壁菌门(86.12%)、变形菌门(12.92%)、放线菌门(0.79%);纯种柯乐猪回肠中的主要菌门为厚壁菌门(97.58%)、变形菌门(0.89%)、放线菌门(0.75%)、无壁菌门(0.52%)。由图 6 可知,乳杆菌属、*Intestinibacter*、*Terrisporobacter*、降解纤维素菌属(*Cellulosilyticum*)为纯种柯乐猪与巴×柯杂交

猪回肠中的优势菌属。由表 3 可知,巴×柯杂交猪回肠中粪球菌属(*Coprococcus*) 的相对丰度显著高于纯种柯乐猪($P<0.05$)。

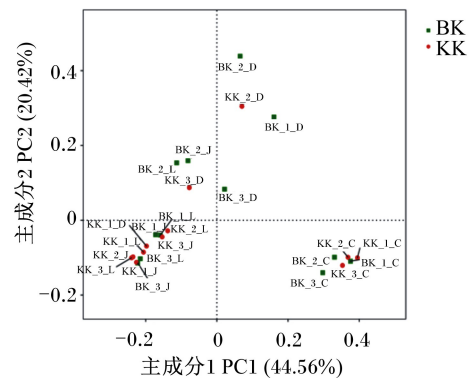


图 4 主坐标分析
Fig.4 PCoA

2.4.4 结肠菌群结构分析

由图 5 可知,厚壁菌门、拟杆菌门、螺旋体门(*Spirochaetae*)为纯种柯乐猪和巴×柯杂交猪结肠中的优势菌门。由表 2 可知,巴×柯杂交猪结肠中

蓝细菌门(Cyanobacteria)的相对丰度显著高于纯种柯乐猪($P < 0.05$)。由图6可知,纯种柯乐猪和巴×柯杂交猪结肠中相对丰度较高的菌属有普氏菌属(*Prevotella*)、链球菌属(*Streptococcus*)、拟普雷沃菌属(*Alloprevotella*)、考拉杆菌属(*Phascolarctobacterium*)、乳杆菌属、密螺旋体属(*Treponema*)、厌氧弧菌属、瘤胃球菌属、粪球菌属、颤螺菌属(*Oscillospira*)、毛螺菌属(*Lachnospira*)。由表3可知,巴×柯杂交猪结肠中脱硫弧菌属(*Desulfovibrio*)的相对丰度极显著高于纯种柯乐猪($P < 0.01$),月形单胞菌属(*Selenomonas*)的相对丰度显著高于纯种柯乐猪($P < 0.05$),消化球菌属(*Peptococcus*)、考拉杆菌属、*Flavonifractor*、聚乙酸菌属(*Acetivibrio*)、*Leeia*的相对丰度显著低于纯种柯乐猪($P < 0.05$)。为了鉴定纯种柯乐猪和巴×柯杂交猪结肠中富集的菌群,对纯种柯乐猪和巴×柯杂交猪结肠中菌群进行LEfSe分析(图7和

图8),结果发现纯种柯乐猪结肠中富集的菌群有厚壁菌门的聚乙酸菌属、厚壁菌门瘤胃球菌科的不可培养瘤胃细菌4C0d_17(*uncultured_rumen_bacterium_4C0d_17*)、变形菌门的 β -变形菌纲(*Betaproteobacteria*)、变形菌门的奈氏球菌科(*Neisseriaceae*)、变形菌门的*Leeia*、厚壁菌门考拉杆菌属的*uncultured_Veillonellaceae_bacterium*、变形菌门的奈氏球菌目(*Neisseriales*)、厚壁菌门的Defluviitaleaceae、拟杆菌门、厚壁球菌门的消化球菌属、拟杆菌门的拟杆菌纲和拟杆菌目、厚壁菌门的氨基酸球菌科(*Acidaminococcaceae*)、厚壁菌门氨基酸球菌科的考拉杆菌属;巴×柯杂交猪结肠中富集的菌群有变形菌门的脱硫弧菌属、厚壁菌门的月形单胞菌属、厚壁菌门瘤胃球菌科的柔嫩梭菌(*Clostridium leptum*)、变形菌门的脱硫弧菌目(*Desulfovibrionales*)和脱硫弧菌科(*Desulfovibrionaceae*)、蓝细菌门。

表2 纯种柯乐猪和巴×柯杂交猪不同肠段菌群门水平相对丰度差异性分析

Table 2 Difference analysis of relative abundance of microflora at phylum level in different intestinal segments between pure-bred Kele pigs and Berkshire×Kele hybrid pigs

菌门	肠道	纯种柯乐猪	巴×柯杂交猪	P 值
Bacteria phylum	Intestine	KK	BK	P-value
拟杆菌门 Bacteroidetes	十二指肠 Duodenum	0.45	5.02	0.041 9
未分类 Unclassified	十二指肠 Duodenum	0.47	4.69	0.129 3
Saccharibacteria	十二指肠 Duodenum	<0.01	<0.01	0.249 1
蓝细菌门 Cyanobacteria	十二指肠 Duodenum	0.01	<0.01	0.283 4
变形菌门 Proteobacteria	十二指肠 Duodenum	9.32	28.16	0.161 4
拟杆菌门 Bacteroidetes	空肠 Jejunum	0.01	0.30	0.187 7
螺旋体门 Spirochaetae	空肠 Jejunum	<0.01	<0.01	0.237 5
变形菌门 Proteobacteria	空肠 Jejunum	0.01	9.81	0.411 4
无壁菌门 Tenericutes	回肠 Ileum	0.53	0.01	0.282 7
梭杆菌门 Fusobacteria	回肠 Ileum	<0.01	0.02	0.348 1
螺旋体门 Spirochaetae	回肠 Ileum	<0.01	<0.01	0.501 8
Saccharibacteria	回肠 Ileum	<0.01	0.04	0.612 0
蓝细菌门 Cyanobacteria	结肠 Colon	0.08	0.21	0.017 5
拟杆菌门 Bacteroidetes	结肠 Colon	42.61	32.90	0.055 0
未分类 Unclassified	结肠 Colon	<0.01	0.05	0.123 5
厚壁菌门 Firmicutes	结肠 Colon	51.92	59.95	0.154 0

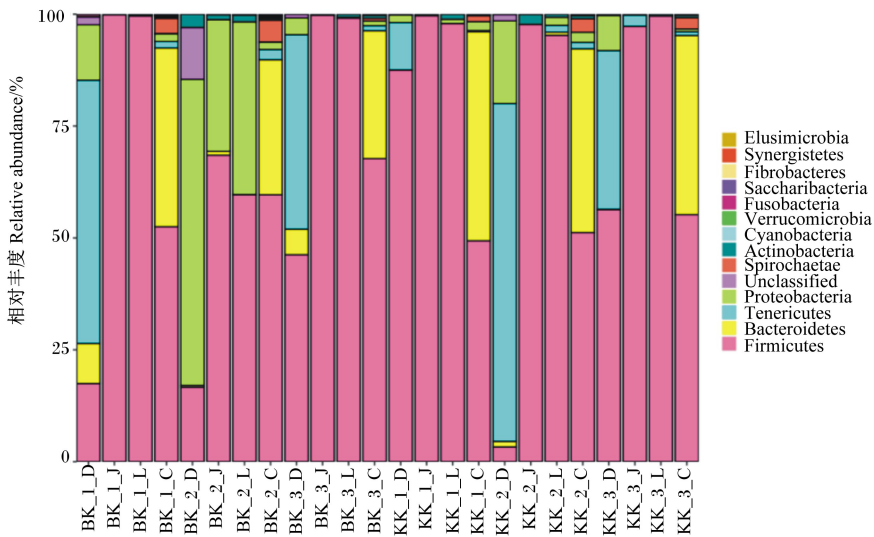
表3 纯种柯乐猪和巴×柯杂交猪不同肠段菌群属水平相对丰度差异性分析

Table 3 Difference analysis of relative abundance of microflora at genus level in different intestinal segment between pure-bred Kele pigs and Berkshire×Kele hybrid pigs

菌属	肠道	纯种柯乐猪	巴×柯杂交猪	P 值
Bacteria genus	Intestine	KK	BK	P-value
克雷伯氏菌属 <i>Klebsiella</i>	十二指肠 Duodenum	<0.000 1	0.017 3	0.006 4
拟杆菌属 <i>Bacteroides</i>	十二指肠 Duodenum	0.017 3	1.060 0	0.012 3

续表 3

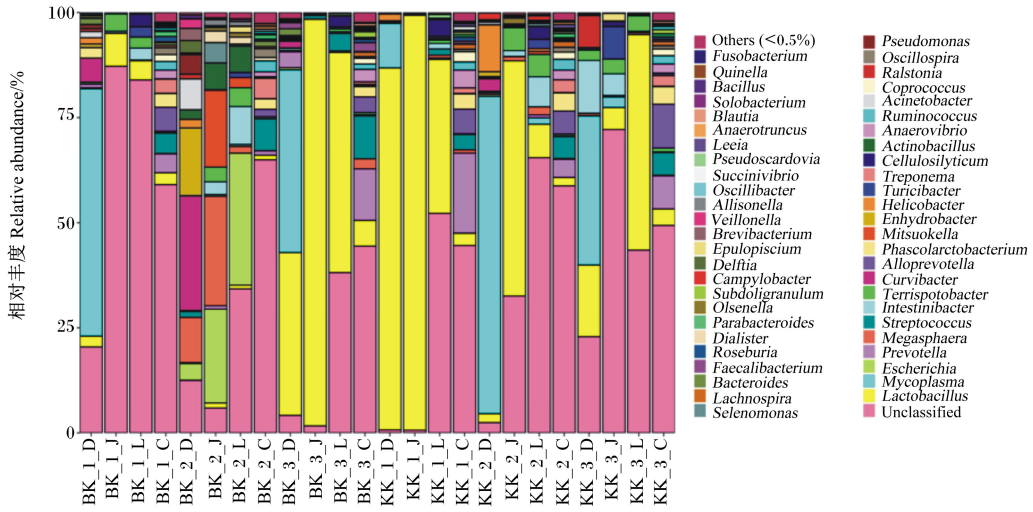
菌属	肠道	纯种柯乐猪	巴×柯杂交猪	P 值
Bacteria genus	Intestine	KK	BK	P-value
艰难杆菌属 <i>Mogibacterium</i>	十二指肠 Duodenum	0.019 5	0.002 1	0.036 0
<i>Pseudoscardovia</i>	十二指肠 Duodenum	0.050 3	0.001 1	0.040 5
孪生球菌属 <i>Gemella</i>	十二指肠 Duodenum	0.016 7	<0.000 1	0.051 4
拟杆菌属 <i>Bacteroides</i>	空肠 Jejunum	<0.000 1	0.007 5	0.010 7
厌氧弧菌属 <i>Anaerovibrio</i>	空肠 Jejunum	<0.000 1	0.007 5	0.019 7
瘤胃球菌属 <i>Ruminococcus</i>	空肠 Jejunum	0.000 8	0.002 8	0.030 5
链球菌属 <i>Streptococcus</i>	空肠 Jejunum	0.080 2	0.442 7	0.100 1
粪球菌属 <i>Coprococcus</i>	回肠 Ileum	<0.000 1	0.004 6	0.039 1
考拉杆菌属 <i>Phascolarctobacterium</i>	回肠 Ileum	<0.000 1	0.008 4	0.064 9
螺杆菌属 <i>Helicobacter</i>	回肠 Ileum	0.335 3	0.000 8	0.087 7
脱硫弧菌属 <i>Desulfovibrio</i>	结肠 Colon	0.017 3	0.208 5	<0.000 1
消化球菌属 <i>Peptococcus</i>	结肠 Colon	0.026 0	0.007 3	0.005 7
考拉杆菌属 <i>Phascolarctobacterium</i>	结肠 Colon	4.028 1	2.729 0	0.017 0
<i>Flavonifractor</i>	结肠 Colon	0.025 8	0.007 4	0.028 0
月形单胞菌属 <i>Selenomonas</i>	结肠 Colon	0.008 9	0.036 8	0.043 0
聚乙酸菌属 <i>Acetitomaculum</i>	结肠 Colon	0.056 9	0.020 2	0.046 7
<i>Leeia</i>	结肠 Colon	0.621 9	0.169 5	0.049 9
毛螺菌属 <i>Lachnospira</i>	结肠 Colon	1.187 1	0.683 8	0.051 3
<i>Moryella</i>	结肠 Colon	0.038 9	0.026 7	0.056 6
拟普雷沃菌属 <i>Alloprevotella</i>	结肠 Colon	7.244 8	3.734 3	0.114 0
粪球菌属 <i>Coprococcus</i>	结肠 Colon	1.896 7	1.253 2	0.123 7



Firmicutes: 厚壁菌门; Bacteroidetes: 拟杆菌门; Tenericutes: 无壁菌门; Proteobacteria: 变形菌门; Unclassified: 未分类; Spirochaetae: 螺旋体门; Actinobacteria: 放线菌门; Cyanobacteria: 蓝细菌门; Verrucomicrobia: 疣微菌门; Fibrobacteres: 纤维杆菌门; Fusobacteria: 梭杆菌门; Synergistetes: 互养菌门; Elusimicrobia: 迷踪菌门。

图 5 纯种柯乐猪和巴×柯杂交猪各肠段菌群门水平相对丰度图

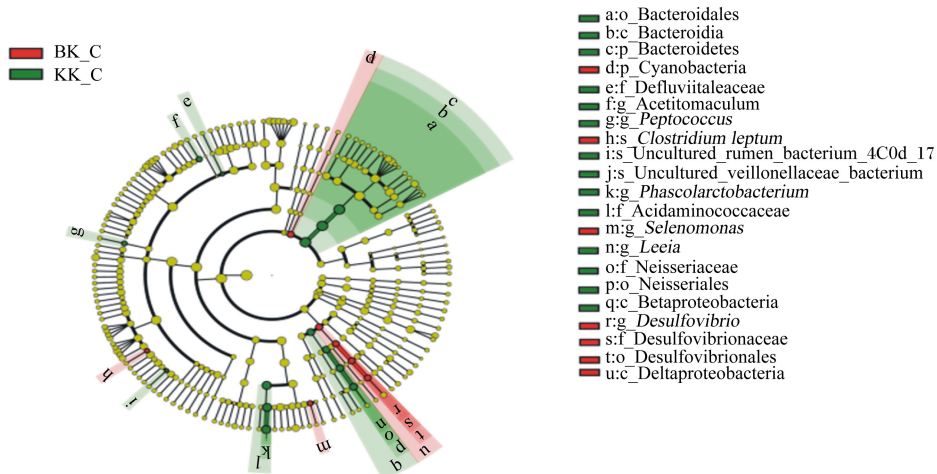
Fig.5 Relative abundance map of microflora at phylum level in each intestinal segment of pure-bred *Kele* pigs and Berkshire×*Kele* hybrid pigs



Unclassified: 未分类; *Lactobacillus*: 乳杆菌属; *Mycoplasma*: 支原体属; *Escherichia*: 埃希菌属; *Prevotella*: 普氏菌属; *Megasphaera*: 巨球型菌属; *Streptococcus*: 链球菌属; *Alloprevotella*: 拟普雷沃菌属; *Phascolarctobacterium*: 考拉杆菌属; *Mitsuokella*: 光冈菌属; *Enhydrobacter*: 栖水菌属; *Helicobacter*: 螺杆菌属; *Turicibacter*: 苏黎世杆菌属; *Treponema*: 密螺旋体属; *Cellulosilyticum*: 降解纤维素菌属; *Actinobacillus*: 放线杆菌属; *Anaerovibrio*: 厌氧弧菌属; *Ruminococcus*: 瘤胃球菌属; *Acinetobacter*: 不动杆菌属; *Coprococcus*: 粪球菌属; *Ralstonia*: 劳尔氏菌属; *Oscillospira*: 颤螺菌属; *Pseudomonas*: 假单胞菌属; *Selenomonas*: 月形单胞菌属; *Lachnospira*: 毛螺菌属; *Bacteroides*: 拟杆菌属; *Roseburia*: 罗氏菌属; *Dialister*: 小杆菌属; *Parabacteroides*: 副拟杆菌属; *Olsenella*: 欧陆森氏菌属; *Subdoligranulum*: 罕见小球菌属; *Campylobacter*: 弯曲杆菌属; *Delftia*: 代尔夫特菌属; *Brevibacterium*: 短杆菌属; *Veillonella*: 韦荣氏球菌属; *Oscillibacter*: 颤杆菌属; *Succinivibrio*: 琥珀酸弧菌属; *Anaerotruncus*: 厌氧棍状菌属; *Fusobacterium*: 梭杆菌属; Others: 其他。

图 6 纯种柯乐猪和巴×柯杂交猪各肠段菌群属水平相对丰度图 (基于相对丰度大于 0.5% 的菌属)

Fig.6 Relative abundance map of microflora at genus level in each intestinal segment of pure-bred Kele pigs and Berkshire×Kele hybrid pigs (based on genus with relative abundance greater than 0.5%)



p: 门 phylum; c: 纲 class; o: 目 order; f: 科 family; g: 属 genus; s: 种 species; *Clostridium leptum*: 柔嫩梭菌; Desulfovibrionales: 脱硫弧菌目; Desulfovibrionaceae: 脱硫弧菌科; Cyanobacteria: 蓝细菌门; Deltaproteobacteria: 变形菌纲; *Selenomonas*: 月形单胞菌属; *Desulfovibrio*: 脱硫弧菌属; Neisseriaceae: 奈氏球菌科; Neisseriales: 奈氏球菌目; Bacteroidetes: 拟杆菌门; *Peptococcus*: 消化球菌属; Bacteroidales: 拟杆菌目; Bacteroidia: 拟杆菌纲; Acidaminococcaceae: 氨基酸球菌科; *Phascolarctobacterium*: 考拉杆菌属。下图同 The same as below。

图 7 多级物种层级树图

Fig.7 Multi-level species hierarchical tree diagram

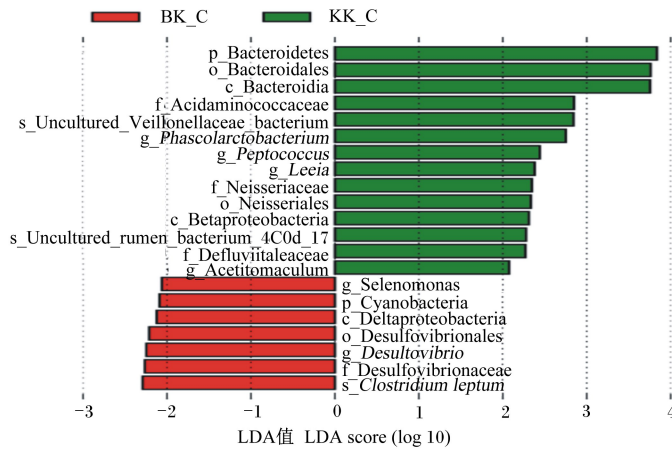


图8 LDA 判别结果图

Fig.8 LDA discrimination result graph

3 讨论

肠道菌群与猪的品种^[9]、年龄^[10]、饲料组成^[11-12]、饲料纤维^[13]、饲养环境^[14]、抗生素^[15]、微生物生态制剂^[16-18]、酶^[19]和植物提取素^[20]等因素有关,也就是说影响肠道菌群的两大主要因素是遗传和环境。相关研究表明,环境因素对人类肠道菌群的影响远大于宿主的遗传背景^[21]。本试验目的是研究纯种柯乐猪和巴×柯杂交猪肠道菌群结构的差异性,为了降低其他因素的影响,选择在相同的饲养环境下饲养,从而突出遗传差异。试验设置重复的主要作用在于估计试验误差和降低试验误差,只有当重复在2个或2个以上的试验上,获得2个或2个以上试验指标的观测值时,才能估计试验误差;误差估计值的大小与重复数的平方成反比,适当增加重复数可以降低试验误差,但在实际应用时,重复数太多,试验动物将随之大大增加,试验动物的初始条件不易控制,也不一定能降低误差。重复数的多少可以根据试验的要求和试验动物的初始条件而定,如果试验动物初始条件差异较大,重复数应多些,若差异较小,重复数可少些。本试验中2个品种猪的心理年龄基本一致,重复数为3头,与相关文献^[22-25]重复数一致。

个体肠道菌群保持相对稳定,同时会随着环境条件的改变而动态变化。随着年龄的增长,并受饲养环境、采食方式以及食物形态等的影响,动物肠道微生物从一个简单的、不稳定的区系逐步发展形成一个稠密的、复杂的区系^[26]。仔猪出生后受到母体产道、粪便以及周围微生物环境的影

响,在胃肠道形成最初的微生物区系,之后随着年龄的增长,最终形成一个稳定的肠道微生物区系定植在猪的胃肠道内^[27]。对纯种柯乐猪和巴×柯杂交猪各肠段菌群的Alpha多样性分析和Beta多样性分析显示2个品种猪肠道菌群丰富度、多样性和结构接近,这可能是因为纯种柯乐猪和巴×柯杂交猪的母本都是柯乐猪以及二者有相同的饲养环境。

肠道菌群结构分析结果显示,纯种柯乐猪和巴×柯杂交猪小肠阶段厚壁菌门、变形菌门、放线菌门占绝对优势,到了结肠阶段变形菌门、放线菌门的比例降低,拟杆菌门的比例相应增加,与厚壁菌门一起构成结肠中的优势菌门,在属水平上乳杆菌属为纯种柯乐猪和巴×柯杂交猪小肠中的优势菌属,与徐娥等^[22]报道一致。

短链脂肪酸(SCFAs)是结肠细胞的重要能量来源,由肠道微生物通过低消化率的碳水化合物发酵而产生^[28-29]。在猪生产养殖中,饲料中的植物性碳水化合物很大一部分不能被小肠消化,而是在大肠中被肠道微生物产生的分解纤维素酶降解,产生短链脂肪酸。本试验中,纯种柯乐猪和巴×柯杂交猪结肠中的优势菌属有普氏菌属、拟普雷沃菌属、密螺旋体属、厌氧弧菌属、瘤胃球菌属、粪球菌属、颤螺菌属、毛螺菌属,这些菌属是纤维降解菌,可通过消化动物体自身不能消化的植物性碳水化合物产生短链脂肪酸^[30-34],与文献^[24,27,36]报道一致。

宿主遗传背景也是影响肠道菌群结构的一个主要因素^[35]。不同品种猪的肠道菌群组成不同,

与杜洛克母猪相比,梅山母猪和二花脸母猪的肠道总细菌、硬毛菌、拟杆菌和减少硫酸盐细菌的数量都更高^[7]。3头不同品种猪(长白、大白、杜洛克)圈养在一起后,其肠道微生物群落变得更加相似,但仍保留一定的品种特异性,长白猪和大白猪的微生物群落与杜洛克猪的微生物群落仍有一定的差异^[37]。对207头体重105 kg的皮特兰猪肠道菌群的研究发现,结肠菌群有着宿主遗传性,其中部分菌属表现出一定的遗传力^[38]。本试验对纯种柯乐猪和巴×柯杂交猪结肠中菌群在属水平上的相对丰度进行了差异性分析,其中脱硫弧菌属在巴×柯杂交猪结肠中的相对丰度极显著高于纯种柯乐猪,月形单胞菌属在巴×柯杂交猪结肠中的相对丰度显著高于柯乐猪,消化球菌属、考拉杆菌属、*Flavonifractor*、聚乙酸菌属、*Leeia*在巴×柯杂交猪结肠中的相对丰度显著低于纯种柯乐猪。月形单胞菌属、聚乙酸菌属也属纤维分解菌,可分解碳水化合物产短链脂肪酸^[39-40],消化球菌属利用蛋白胨或氨基酸为能源产生挥发性脂肪酸,部分可发酵碳水化合物产挥发性脂肪酸^[41]。相关研究表明,相对于猪的不同膳食纤维成分,在补充果胶的情况下,肠道中*Leeia*的比例更高^[42],说明*Leeia*对粗纤维有一定的降解能力。拟杆菌属和普氏菌属可降解动物体内不能被吸收消化的粗纤维产生琥珀酸盐,而考拉杆菌属利用琥珀酸盐作为碳源产生短链脂肪酸为机体提供营养^[43-44]。这些结肠中的大量纤维降解菌与纯种柯乐猪对粗纤维的消化能力息息相关。对纯种柯乐猪和巴×柯杂交猪结肠中菌群进行LEfSe分析,发现纯种柯乐猪结肠中富集的菌群有厚壁菌门的聚乙酸菌属、厚壁菌门瘤胃球菌科的 *uncultured_rumen_bacterium_4C0d_17*、变形菌门的 *Leeia*、厚壁菌门考拉杆菌属的 *uncultured_Veillonellaceae_bacterium*、拟杆菌门、厚壁球菌门的消化球菌属、拟杆菌门的拟杆菌纲和拟杆菌目、氨基酸球菌科、考拉杆菌属。这些细菌中,除上文提到的纤维分解菌外,瘤胃球菌科的 *uncultured_rumen_bacterium_4C0d_17* 也属于纤维分解菌^[45-46];此外,氨基酸球菌科则是主要利用氨基酸作为能源产生乙酸和丁酸^[47]。巴×柯杂交猪结肠中富集的菌群有厚壁菌门的月形单胞菌属、产丁酸的柔嫩梭菌^[48],可以看出巴×柯杂交猪结肠中纤维降解菌的比例较弱于纯种柯乐猪。

4 结 论

① 在整个菌群结构上,纯种柯乐猪和巴×柯杂交猪肠道中优势菌在类别上非常接近,体现了环境相同效应,纯种柯乐猪和巴×柯杂交猪的肠道菌群结构相对稳定且相似度高。

② 纯种柯乐猪对粗纤维的消化能力强与肠道内含有大量的纤维降解菌(普氏菌属、厌氧弧菌属、瘤胃球菌属、颤螺菌属等)有关。

③ 引入外源血影响了肠道菌群结构,巴×柯杂交猪对粗纤维的消化能力较弱于纯种柯乐猪。

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Study on Intestinal Microflora Structure of Pure-Bred *Kele* Pigs and Berkshire×*Kele* Hybrid Pigs

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Abstract: This experiment studied the structural characteristics and differences of intestinal microflora of pure-bred *Kele* pigs and Berkshire×*Kele* hybrid pigs, aiming to reveal the rough feed tolerance characteristics of *Kele* pigs and the ability to adapt to green feed of Berkshire×*Kele* hybrid pigs from the perspective of pig gut microbes, and to provide a basis for the design of microbial additives in the later stage, the cross-utilization of *Kele* pigs and standardized breeding. Pure-bred *Kele* pigs and Berkshire×*Kele* hybrid pigs were raised under the same conditions. After reaching the slaughter weight (about 100 kg), three pigs were randomly selected for slaughter, and the contents of the duodenum, jejunum, ileum and colon were taken to perform high-throughput sequencing and biological information analysis. The results showed that there were no significant differences in Alpha diversity indexes in each intestinal segment between Berkshire×*Kele* hybrid pigs and pure-bred *Kele* pigs

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($P>0.05$). In Beta diversity analysis, most samples in the two breeds of pigs crossed and gathered together. Intestinal microflora structure analysis showed that, Firmicutes, Proteobacteria, Actinobacteria were the dominant bacterial phyla in the stage of small intestine of Berkshire×Kele hybrid pigs and pure-bred Kele pigs, and Bacteroidetes and Firmicutes were the dominant bacterial phyla in the stage of colon. There were a lot of cellulose decomposition microbes in the colon of Berkshire×Kele hybrid pigs and pure-bred Kele pigs. The relative abundances of *Selenomonas* and *Desulfovibrio* in the colon of Berkshire×Kele hybrid pigs were significantly higher than those of pure-bred Kele pigs ($P<0.05$ or $P<0.01$), and the relative abundances of *Peptococcus*, *Acetitomaculum* and *Leeia* were significantly lower than those of pure-bred Kele pigs ($P<0.05$). LEfSe analysis found that a lot of short chain fatty acid producing bacteria enriched in the pure-bred Kele pigs' colon. Among them, *Acetitomaculum*, uncultured_rumen_bacterium_4C0d_17, *Leeia*, Bacteroidia and Bacteroidales belonged to fiber-degrading bacteria, and some bacteria had synergistic effects with the fiber-degrading bacteria, they were *Phascolarctobacterium* and uncultured_Veillonellaceae_bacterium. The fiber-degrading bacteria such as *Selenomonas* and *Clostridium leptum* enriched in the Berkshire×Kele hybrid pigs' colon. Above results indicate that, in the structure of entire intestinal microflora, the type of dominant bacteria in pure-bred Kele pigs and Berkshire×Kele hybrid pigs is almost similar, which reflects the same environmental effects, and the structure of the intestinal microflora of pure-bred Kele pigs and Berkshire×Kele hybrid pigs is relatively stable and the similarity is high; at genus level, the fiber-degrading bacteria show some differences between pure-bred Kele pigs and Berkshire×Kele hybrid pigs, and fiber-degrading bacteria accounts for a higher proportion in cecal microflora of pure-bred Kele pigs, which indicates that the introduction of exogenous blood has a certain impact on the intestinal microflora, and Berkshire×Kele hybrid pigs are weaker in digesting crude fiber than pure-bred Kele pigs. [*Chinese Journal of Animal Nutrition*, 2021, 33(3):1359-1371]

Key words: pure-bred Kele pigs; Berkshire×Kele hybrid pigs; intestinal microflora; high-throughput sequencing; bioinformatic analysis