

Effects of Steam-Flaked Grains On Foals Growth Performance And Faecal Microbiota

XiaoBin Li

Xinjiang Agricultural University

Xin Xin Huang

Xinjiang Agricultural University

Chang Jiang Zang

Xinjiang Agricultural University

Chen Ma

Xinjiang Agricultural University

Kai Xu Chen

Xinjiang Agricultural University

Guo Dong Zhao

Xinjiang Agricultural University

Qian Li

Xinjiang Agricultural University

Xuan Yue Li

Xinjiang Agricultural University

Wen Jie Zhang

Xinjiang Agricultural University

Kai Lun Yang (✉ 1196129362@qq.com)

Xinjiang Agricultural University

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Abstract

Background

There is little objective information concerning the effect of steam-flaked grains on foal's growth performance and faecal microbiota. To determine the effects of steam-flaked grains on foal's growth performance and faecal microbiota, faecal samples were collected from 18 foals which had been fed corn, oat or barley diets over the 60 days of the experiment. Body weight and measurements were collected. Next-generation sequencing of the V3+V4 region of the 16S rRNA gene was used to assess the microbial composition of faeces. Alpha diversity, Venn graph, Relative abundance and beta diversity are presented.

Results

There was a significantly higher increase in the body weight of those foals fed barley compared to either corn or oats, both in terms of the total weight gain and the daily weight gain ($P=0.0185$). There were also significant changes in the Alpha diversity. The Shannon and Simpson indices were higher in the barley fed group than those fed corn or oats ($P<0.05$, $P<0.05$, $P<0.05$ and $P<0.05$). The Chao1 index was higher in the oat fed group than the corn or barley fed groups ($P<0.05$ and $P<0.05$). There were significant changes in the relative abundance of bacteria in the microbiota in terms of phylum, family and genus. The histogram of LDA value distribution showed that the statistically different biomarkers of the bacteria was 12. Tax4Fun function annotation clustering heat map showed that functional information was detected from 26 species of bacteria in faecal samples from the foals.

Conclusions

Differences were seen in the faecal microbiota of foals fed either corn, oat or barley, and also differences in the overall growth of the foals. Different grains have different impact on faecal microbiota, which are mainly related to the grain sources. Further investigation is required to look at the potential impact of changes in the microbiota on the functional impact on foals when fed grains.

Highlights

1. To improve the efficiency of grain feeding to equine and prevent diseases and health problems associated with the fermentation of starch in the hindgut, and must be enhanced digestibility of starch in the small intestine.
2. In current study, studies have not investigated how starch source and processing affects equine growth and hindgut microbiota. We hypothesized that changes to the hindgut bacteria in response to dietary starch would be affected by the processing of starch. The objective was to compare the effects of adding oats, corn or wheat middlings to a forage-based diet on equine hindgut microbiota.
3. There is little objective information concerning steam-flaked grains on foals growth performance and faecal microbiota.

4. Different grains have different impact on faecal microbiota, which are mainly related to grain sources.

Introduction

The gut microbiota performs essential roles in the maintenance of growth and health of animals (Marcio et al.,2015). In equine species, the gut bacterial microbiota is essential due to its role in cellulose fermentation and short-chain fatty acid production as the primary energy sources for growth and health (Glinsky et al.,1976). Equines are able to graze on high-fibre, low-energy fodder due to the complex microbial community in their gut. However, factors such as diet (Daly et al.,2012), age, management, gut disease (Costa et al.,2012) and weaning have all been shown to cause changes in the gut microbiota of equines. Corn, oat, and barley are often included in equine diets to increase energy (Svihus et al.,2005). They have similar amylose and amylopectins, but differ in the proportion of those polysaccharides, and also in the morphology of the starch granule (Tester et al.,2006). In the small intestine, the starch of grains is digested by amylolytic enzymes and absorbed as glucose. Any starch which is not digested in the small intestine of the equine will be delivered to the caecum and colon, and fermented by bacterial microbiota. In the hindgut, starch fermentation can lead to increased numbers of amylolytic bacteria, including lactobacilli and streptococci. This can increase the volatile fatty acid and lactic acid concentrations, decrease pH, and decrease the number of cellulolytic bacteria (Defombelle et al.,2001; Medina et al.,2002; Willing et al.,2009) To improve the efficiency of grain feeding to equines and to prevent diseases and health problems associated with the fermentation of starch in the hindgut, the digestibility of starch in the small intestine must be enhanced. Currently, the digestibility of starch in the small intestine is enhanced mainly through the selection and processing of grains. The purpose of grain processing is to enhance digestibility in the small intestine by changing its physical and chemical structure. Processing methods utilizing a combination of heat, moisture and pressure can disrupt the starch granule structure, destroy crystalline starch formations, increase the water solubility of starch and physically expose starch to digestive enzymes (Kienzle et al.,1997; Rowe et al.,1999). Steam-pressed tableting is the most commonly used process for starch. Generally, grains are processed by steam at 100–110°C for 30 to 60 minutes. The grains are then ground into particles of specific density by a pair of reverse rotating preheated rollers, which are dried and cooled to achieve safe water storage. Steam-pressed tablets made from grain improve the digestibility of starch in the small intestine. However, studies have not investigated how the source of the starch and processing affects equine growth and hindgut microbiota. We hypothesized that changes to the hindgut bacteria in response to dietary starch would be affected by the processing of starch. The objective was to compare the effects of adding oats, corn or wheat middlings to a forage-based diet on equine hindgut microbiota.

Materials And Methods

Animals

Eighteen healthy 5-month-old weaning Kazakh foals with a starting bodyweight of 112.36 ± 7.50 kg were studied. The foals were born in March, weaned in August, and the trial undertaken between August and October(60 days). Foals were selected from the same local pasture and were clinically normal with no history of systemic illness. Ivermectin was used for deworming the foals before weaning.

Foals were fed the same alfalfa, dry hay and concentrate supplement. Starch was introduced into the diet from steam-pressed corn, steam-pressed oats or steam-pressed barley. All foals were randomly allocated to one of the 3 treatments: corn group, oat group, or barley group (6 foals per group). The sum of starch in each starch source was used to adjust feed amounts to provide 2 g starch/kg bodyweight (Dry Matter,DM basis). The feed amount of the concentrate supplement was calculated according to the feed amount of the starch. The foal weighed 112 kg at 0–30 days and required 224 g of starch. The average weight of the 31–60 day old foals was 126 kg and the starch requirement was 252 g (Tables 1& 2).The nutrient levels of the foal's diet during the test period was shown in Table 3.

Table 1
Supplementary feeding amount of concentrate supplement

Groups	0–30 d(g)	31–60 d(g)
Corn	650	710
Oat	660	720
Barley	660	720

Table 2
Composition of concentrate supplement(%)

The raw material	Corn group	Oat group	Barley group
Steam-pressed corn	60	–	–
Steam-pressed oats	–	66	–
Steam-pressed barley	–	–	66
Soybean meal	36	30	30
Calcium hydrogen phosphate	2	2	2
Limestone	1	1	1
Premix*	0.5	0.5	0.5
Salt	0.5	0.5	0.5

*The premix provided the following per kg of concentrate supplement: Vitamin A 3000 IU,Vitamin B₁ 20 mg,Vitamin B₂ 20 mg,Vitamin B₆ 6 mg,Vitamin C 20mg,Vitamin D 1000 IU,Vitamin E 500 IU, Pantothenic acid 10 mg, Nicotinamide 100 mg, Cu 25 mg, Fe 107 mg, Mn 81 mg, Zn 74 mg, I 6 mg, Se 14 mg,Co3 mg, Choline chloride 120 mg.

Table 3
Nutrient levels of hay, alfalfa and concentrate Supplements (Dry Matter,DM basis)

Nutrient	Hay	Alfalfa	Corn group	Oat group	Barley group
Dry Matter,DM (%)	89.75	87.56	92.44	93.28	91.93
Organic Matter,OM (%)	91.80	92.21	94.46	93.32	93.90
Crude Protein,CP (%)	10.75	20.56	26.26	26.09	26.86
Gross Energy,GE (MJ/kg)	18.93	19.53	18.25	18.29	18.26
Starch (%)	3.63	4.46	37.68	37.00	38.27
Neutral Detergent Fiber,NDF (%)	60.00	52.60	24.46	27.04	24.79
Acid Detergent Fiber,ADF (%)	41.82	45.21	5.10	9.09	5.71
Calcium,Ca (%)	0.33	1.50	1.06	1.45	1.14
Phosphorus,P (%)	0.27	0.25	0.94	1.02	1.02

Management

During the 60 days study, the foals were housed in individual, partially covered runs with red brick (8×6 m). Each run contained an automatic water source and the feeding area of the run was equipped with red brick, and a large wood tub secured to the wall. Horses were allowed turnout per day in dry lot paddocks after feeding.

Weight and body measure data collection

The body weight of foals was assessed using a loadometer (range 1000 kg), the body height and length were taken using a measuring rod, and the chest and shank circumference were taken using a tape measure (range 5 m). The data were collected before the first feed in the morning on the first, thirtieth, and sixtieth day of treatment.

Faecal sample collection

Faecal samples were collected by rectal palpation using one rectal sleeve per foal. Samples were stored in plastic sterile containers (RNA free) and snap frozen in liquid nitrogen before storage at -70°C until DNA extraction. Samples were collected before the first feed in the morning on the sixtieth day of treatment.

DNA extraction

Total genome DNA from samples was extracted using a CTAB/SDS method (Magoč et al.,2011). DNA concentration and purity was assessed on 1% agarose gels. DNA was diluted to 1 ng/μl using sterile water.

16 s rRNA gene amplification and sequencing

PCR amplification of the V3 + V4 region of 16S rRNA gene was undertaken. The primers used for the amplification were 341F: CCTAYGGGRBGCASCAG and 806R: GGACTACNNGGGTATCTAAT.

16S rRNA genes were amplified using the specific primer with the barcode. All PCR reactions were carried out in 30µL reactions with 15 µL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs); 0.2 µM of forward and reverse primers, and approximately 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s. Finally 72°C for 5 min (Magoč et al.,2011; Caporaso et al.,2010; Wang et al.,2007).

Sequencing libraries were generated using Illumina TruSeq DNA PCR-Free Library Preparation Kit (Illumina, USA) following manufacturer's recommendations, and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina NovaSeq platform and 250 bp paired-end reads were generated.

Paired-end reads from the original DNA fragments are merged by using FLASH.

(1) Paired-end reads was assigned to each sample according to the unique barcodes. Sequences were analyzed using QIIME (Caporaso et al.,2010; McDonald et al.,2012).

(2)Software package (Quantitative Insights into Microbial Ecology), and in-house Perlscripts were used to analyze alpha- (within samples) and beta- (among samples) diversity. First, reads were filtered by QIIME quality filters. Sequences with $\geq 97\%$ similarity were assigned to the same OTUs. A representative sequences for each OTU was picked, and used as the RDP classifier(Rideout et al.,2014; Cole et al.,2009).

(3) In order to compute Alpha Diversity, we rarified the OTU table and calculated three metrics: Chao1 estimates the species abundance, Observed Species estimates the number of unique OTUs found in each sample, and the Shannon index. Rarefaction curves were generated based on these three metrics. QIIME calculates both weighted and unweighted unifrac, which are phylogenetic measures of beta diversity. To mine deeper data of microbial diversity of the differences between the samples, significance tests were conducted with some statistical analysis methods, including T-test, MetaStat, LEfSe, Anosim and MRPP(Segata et al.,2011; Langille et al.,2013).

Data analysis

Weight and body measure data analysis

Data were analyzed by Statistical Analysis System(SAS) (2013) as a randomized block design, considering the starch source as the main effect and the replicate as a block. The MIXED procedure was used to analyze body weight and body measurements. The variance structure adopts CS(Compound Symmetry), the data is the least square mean value, the significance judgment standard is $P < 0.05$, and the Ls means method is used for multiple comparison.

Alpha diversity and relative abundance analysis

Statistical analyses on the microbial dataset were performed in R. Diversity and richness for both diets were evaluated using the Shannon diversity and Chao1 richness indices. T-test, LEfSe, and Anosim statistical analysis methods were used to test the significance of differences in the species composition and community structure of the group samples. Tax4Fun was used to predict and analyze the microbial community in the samples. To investigate microbial community structure of faecal in foals.

Results

Growth performances

All foals remained in good health without diarrhea, stress or intestinal inflammation throughout the treatment. All foals adapted to the treatment diets and feeding management without problems. Table 4 shows the effect of the grain source on the growth performance of foals. The weight and body size least-square results of the foals during the experimental period showed that the body weight of the foals in the barley fed group was significantly higher than that of the oat fed group ($P=0.0152$), and the effects of barley and corn supplementation on the body weight and body size of the foals were better than that of the oat group. However, after supplementing with corn, oats, or barley, weight and body size changed significantly as the foal aged ($P<0.01$). There were no differences amongst the treatments in body height, body length, chest and shank circumference of foals ($P>0.05$).

Table 4
The effect of the source of grains on growth performances of foals

Item	corn	oat	barley	SEM	P value		
					trt	date	trt×date
Body weight (kg)	127.33 ^{ab}	125.05 ^b	130.28 ^a	1.5230	0.0509	<.0001	0.4238
Body height (cm)	117.61	116.82	117.44	1.1757	0.8707	0.0018	0.5372
Body length (cm)	114.46	112.57	114.06	1.4039	0.5803	<.0001	0.7843
Chest (cm)	110.99	109.81	112.06	1.4058	0.5061	<.0001	0.7567
Shank circumference (cm)	14.56	14.20	14.46	0.1862	0.3355	<.0001	0.7979

The total and average daily increases in body weight and body size of the foals are shown in Fig. 1. The source of grains could effect the total and average daily increases in body weight and body size of the foals. The total weight gain and daily weight gain of the barley fed group was significantly higher than those foals fed corn or oats ($P=0.0185$). There were no differences between the treatment groups in terms of body height, body length, chest or pipe circumference ($P>0.05$).

Alpha Diversity indices

Alpha Diversity was used to analyze the microbial community diversity within the faecal samples (Li et al., 2013). The single-sample diversity analysis (Alpha Diversity) could reflect the richness and diversity of the microbial community in the sample, including a series of statistical analysis indices to evaluate the differences in the species richness and diversity of the microbial community in each sample. Alpha diversity indices of the faecal bacteria are shown in Table 5. There were no significant differences in PD(Compute Faith's phylogenetic diversity metric)_whole_tree index, ace index, or goods coverage of all groups. There were significant differences in observed species index, Shannon index, Simpson index and Chao1 index of all groups. The observed species index was higher with the oat fed group than the corn fed group ($P < 0.05$). The Shannon and Simpson indices were higher in the barley fed group than in the corn or oat fed groups ($P < 0.05$, $P < 0.05$, $P < 0.05$ and $P < 0.05$). The Chao1 index was higher in the oat group than either the corn or barley group ($P < 0.05$ and $P < 0.05$).

Table 5
Alpha diversity indices of fecal microbiota

Items	corn	oat	barley
Observed_species	1424.40 ± 52.68 ^b	1561.17 ± 179.55 ^a	1447.20 ± 60.76 ^{ab}
PD_whole_tree	102.41 ± 18.68	120.05 ± 21.92	102.60 ± 31.88
Shannon	6.74 ± 0.26 ^b	6.96 ± 0.61 ^b	7.56 ± 0.51 ^a
Simpson	0.96 ± 0.01 ^b	0.95 ± 0.03 ^b	0.98 ± 0.01 ^a
Chao1	2291.39 ± 214.16 ^b	2269.51 ± 163.55 ^a	2133.51 ± 102.39 ^b
ace	1729.13 ± 77.04	1767.14 ± 68.99	1727.12 ± 77.54
Goods_coverage	0.99 ± 0.00	0.99 ± 0.00	0.99 ± 0.00

Venn graph based on operational taxonomic units

The Venn diagram (Fig. 2) shows the three different feeds and the number of common OTUs between the groups, and the number without overlap which represent the unique OTUs of the group. The common OTUs of the three groups is 1745. The number of unique OTUs is 311 in the corn fed group, 383 in the oat fed group, and 356 in the barley fed group. The number of unique OTUs in the oat fed group is higher than in the corn or barley fed and suggests that more bacterial species were detected in the oat group.

Relative abundance of faecal bacteria

The relative abundance of faecal bacteria at the phylum (A), family (B), genus (C) and species (D) levels are shown in Fig. 3. The top ten bacteria found in the faecal samples at the phylum level were Firmicutes, Bacteroidetes, Verrucomicrobia, Euryarchaeota, Spirochaetes, Proteobacteria, Fibrobacteres, Kiritimatiellaeota, Tenericutes, and Actinobacteria. The majority of bacteria found in all faecal samples throughout the treatment were assigned as the phyla Firmicutes (corn, oat and barley; 47.72%, 56.65% and 49.87%, respectively) and Bacteroidetes (corn, oat and barley; 29.39%, 27.44% and 36.03%,

respectively). *Tenericutes* was significantly more abundant in samples from oat fed foals compared to the corn fed foals ($P = 0.027$). Actinobacteria was significantly more abundant in corn fed foal's samples compared to the barley fed group ($P = 0.028$).

At the family levels, the 10 top bacteria were Lactobacillaceae, Akkermansiaceae, Ruminococcaceae, Lachnospiraceae, Rikenellaceae, Streptococcaceae, Methanocorpusculaceae, Prevotellaceae, Spirochaetaceae and Christensenellaceae. There were significant differences in relative abundance, with Rikenellaceae more abundant in the oat fed group compared to the corn fed group ($P = 0.039$). The Streptococcaceae were more abundant in the corn fed group compared to the oat group and barley fed groups ($P = 0.001$ and 0.010). The Lactobacillaceae were more abundant both in the corn and oat group compared to the barley group ($P = 0.039$ and 0.001). The Lachnospiraceae were more abundant in the barley fed group compared to the corn fed group ($P = 0.023$).

At the genus levels, there were significant differences in the relative abundance of the top 20 bacteria. *Streptococcus* was more abundant in the corn fed group compared to the oat and barley fed groups ($P = 0.012$ and 0.015). *Lactococcus* was more abundant in the corn fed group compared to the oat and barley fed groups ($P = 0.021$ and 0.002), and the barley fed group compared to the oat fed group ($P = 0.006$). *Actinobacillus* was more abundant in the corn fed group compared to the oat and barley fed groups ($P = 0.002$ and 0.013). *Lactobacillus* was more abundant both in the corn and oat fed groups compared to the barley fed group ($P = 0.049$ and 0.010). *Agathobacter* was more abundant in the barley fed group compared to the oat fed group ($P = 0.041$).

At the species levels, there were 20 top bacteria shown: *Lactobacillus hayakitensis*, *Lactobacillus equigenosus*, *Escherichia coli*, *Bacteroidales bacterium Bact 22*, *Treponema sp 9:A:D01*, *Clostridium butyricum*, *Treponema saccharophilum*, *Lactobacillus equi*, *Clostridium sp ID5*, *Ruminococcus sp HUN007*, *rumen bacterium NK4A214*, *Methanobrevibacter ruminantium*, *Weissella cibaria*, *Ruminococcus flavefaciens*, *Clostridium perfringens*, *Streptococcus salivarius subsp salivarius*, *Butyrivibrio sp oral clone DA074*, *Fibrobacter sp*, *Actinobacillus pleuropneumoniae*, and *Sphingomonas aerolata*.

LDA Effect Size

LEfSe (LDA Effect Size) (Segata et al., 2011) is an analytical tool for the discovery and interpretation of high-dimensional biomarkers, which can be used for comparison between two or more groups. It emphasizes statistical significance and biological correlation, and can be used to find statistically different biomarkers between groups. Figure 4 shows the characteristics of different abundance and associated categories of the results. LEfSe's statistical results include three parts, which are (1) the histogram of LDA value distribution, (2) the evolutionary branch (phylogenetic distribution), and (3) the abundance comparison of biomarkers with statistically significant difference among different groups. The histogram of LDA value distribution (Fig. 4A) shows that the statistically different biomarkers bacteria were Bacilli, Lactobacillales, Lactobacillaceae, *Lactobacillus* and *Lactobacillus hayakitensis* in the oat fed group; the Streptococcaceae, *Streptococcus* and Proteobacteria in the corn fed group; and the Clostridia, Clostridiales, Lachnospiraceae and Ruminococcaceae in the barley fed group. The evolutionary

branch (phylogenetic distribution) and the abundance comparison of biomarkers showed that eight bacteria were Lactobacillaceae, Streptococcaceae, Lactobacillales, Bacilli, Lachnospiraceae, Ruminococcaceae, Clostridiales and Clostridia in all groups, respectively (Fig. 4B).

Function prediction of faecal bacteria

Tax4Fun (Aßhauer et al.,2015) is an R package based on 16S Silva database for predicting the function of intestinal and soil environmental samples. The prediction accuracy is high, and the experimental results show that the PICRUST function prediction is better than the PICRUST function prediction, especially suitable for soil and other complex environment samples. The function prediction of Tax4Fun was realized by using the nearest neighbor method based on the minimum 16S rRNA sequence similarity. Specifically 16S rRNA gene sequences were extracted from the whole genome of prokaryotes in the KEGG database and compared to the SILVA SSU Ref NR database using BLASTN algorithm (BLAST Bitscore & GT;1500). The established correlation matrix corresponded to the whole genome function information of prokaryotes annotated through UProC and PAUDA to SILVA database, and realized the function annotation of SILVA database. The sequencing samples cluster OTU with the SILVA database sequence as the reference sequence, and then obtained the functional annotation information. Tax4Fun functional annotation clustering heat map of foal faecal samples are shown in Fig. 5. We detected functional information of 26 species of bacteria in foal faecal samples. There were 7 significantly increased species or functional information in the barley fed group: nitrogen fixation, ark hydrogen oxidation, others, ark hydrogen oxidation, reductive acetogenesis, xylanolysis, methanogenesis by reduction of methyl compounds with H₂, methylotrophy and aerobic chemoheterotrophy. In the corn fed group, we detected four species functional information which were significantly increased: aerobic chemoheterotrophy, cellulolysis, animal parasites or symbionts and nitrate reduction. There were three species functional information which were significantly increased in the oat fed group: methanogenesis by CO₂ reduction with H₂, methanogenesis, and hydrogenotrophic methanogenesis.

Discussion

Starch is the storage form of plant nutrients. Grains contain a large amount of starch, which generally accounts for 20% ~ 40% of the dry matter of the diet. Most starches in grains are found in the endosperm, which contains about 70 %. Starch is a chain polymer composed of many glucose molecules linked together, divided into amylose and amylopectin. The average molecular weight of amylose was 1×10^5 to 9×10^6 (Doblado Maldonado et al.,2017), and the average molecular weight of amylopectin was 1×10^7 to 1×10^9 , being some of the largest polymers in nature (Tester et al.,2004). During the formation of starch granules, amylose penetrates among amylopectin, forming dense hydrogen bonds, preventing the invasion of digestive enzymes, and reducing starch fermentation (Gómez et al.,2016). The higher the ratio of amylose to amylopectin, the lower the digestibility of starch (Svihus et al.,2005). Therefore, an increase in amylose ratio will make starch degradation more difficult.

The steam sheet is a common heat treatment for grain. The process not only utilizing the grain, but also changes its crystal. The structure makes cereal starches easier to digest in the small intestine. Perez *et al.* (2010) believed that compared with crushing treatment, steam compression treatment could achieve complete utilizing and increase the utilizing rate of starch. Meyer *et al.*(1993) reported the effects of varying grain processing methods on the pre-caecal starch digestion of corn in horses. The results showed that whole corn, cracked corn, ground corn, and popped corn pre-caecal digestibility were 28.9%, 29.9%, 45.6% and 90.1%, respectively. A study by Mohsen *et al.*(2020) showed that the average daily gain and feed efficiency were improved and final body weight was higher in steam-flaked corn grain-fed calves.

In our study, we showed that 2 g starch/kg BW day (DM basis) could improve the body weight gain of foals. In addition to the different sources of starch, the composition and nutritional composition of other feeds were the same, as well as the feed processing method and the breeding and management conditions of foals. Thus, differences in dietary nutritional effects can be attributed to the source of starch. Among the three kinds of starches, the composition of starches is different, among which the proportion of amylose and amylopectin was different. A study showed that the ratio of amylose to amylopectin could effect growth performance and pork quality in pigs. Potter *et al.*(1992) suggested that the upper limit of starch digestion in the equine small intestine was between 3.5 and 4 g starch/kg body weight per feeding^[24]. However, others have suggested that in order to limit starch bypass to the large intestine that starch intake should not exceed 2 g/kg body weight per meal (Meyer *et al.*,1995; Kienzle *et al.*,1992). In this study, no digestive abnormalities were observed in the foals, indicating that the increased starch content in this study met the needs of the foals. The total weight gain and daily weight gain of the barley fed group was significantly higher than that of the corn and oat fed groups. The uniform and stable release of glucose by the body is a necessary condition for the absorption and utilization of glucose in the small intestine to promote the growth and development potential of young animals. Only when the release of glucose in the intestine is consistent with the demand for glucose by the body tissues and organs can the performance potential of young animals be fully achieved. Results of Weurding *et al.*(2001), showed that a continuous, uniform and slow-release mode of glucose provided energy supply for the balanced intestinal tract of piglets, and allowed their growth to reach the optimal level. A study by Nasir *et al.*(2015) showed that low-quality or high-quality barley can fully replace wheat grain in diets for starter pigs and achieve equivalent or better growth performance. In this study, the significant weight gain in the barley fed group may be related to the speed of starch release from barley; releasing glucose more in line with the growth pattern of the foals.

The grain in the diet was used mainly to increase the energy requirements of the horse. Corn, oats and barley were the primary energy feed for horses, which contain similar amylose and amylopectin. However, due to the different proportion of starch polysaccharides and the different size of starch granules, their digestion and effects on the intestinal tract of horses are other (Kong *et al.*,2003; Svihus *et al.*,2005). Studies have shown that there were differences in the digestibility of grain particles in the small intestine

of horses. Starch that was not digested in the small intestine was degraded by microbial fermentation upon arrival in the large intestine (Defombelle et al., 2004).

The starch that is not digested by enzymes in the horse's small intestine is transported to the hind intestine for microbial fermentation. It has been estimated that the utilized energy of starch produced by post-intestinal microorganisms is 35% – 40% less than that absorbed in the form of glucose digested by enteroglycans (Kienzle et al., 1994; Black et al., 1971). Also, starches not digested by intestinal amylase arrive in the large intestine. They are fermented by starch-breaking bacteria, causing fundamental changes in the structure of the posterior intestinal flora and increasing concentrations of volatile fatty acids and lactic acid in the caecum and colon (Garner et al., 1978).

Different starch sources and other intakes of starch would affect the number of starch decomposition bacteria. The amount of grain the foals were fed determined the amount of starch that they consumed. When fed a low-content grain diet, the starch intake was low, and the number of starch decomposition bacteria increased in horses fed with corn and wheat, but not in horses fed with oats (Harlow et al., 2016). When the amount of grain was high, the number of starch-breaking bacteria increased in horses fed with oats and corn, while the increase of starch-breaking bacteria was more significant in horses fed with a high corn diet. Such a massive difference in microbial digestion of corn starch and oat starch is directly related to the low sensitivity of corn starch to enzymic digestion in the small intestine of horses (Rosenfeld et al., 2008). As alpha-amylase in the small intestine of horses showed low activity in the digestion of corn starch, a large amount of undigested corn arrived in the large intestine. This was fermented by microorganisms, increasing starch-breaking bacteria (Harlow et al., 2015). Therefore, the varying starch sources could explain the influence of different starch sources on the microbial diversity of the hindgut, especially on the total starch decomposers. The starch source has been shown to affect the extent of the starch bypass to the equine hindgut (Radicke et al., 1991; Defombelle et al., 2004; Rosenfeld et al., 2008). The starch source would also affect changes to the gastrointestinal microbial community that are induced when the grain is added to a forage-based diet. Ren *et al.* (2019) used 16S rRNA sequencing technology to reveal the potential mechanism of steam-pressed corn to improve the production performance of ruminants, and fed steam-pressed corn to cows. They subsequently found Firmicutes and mutants in the rumen microbial community. The relative abundance of the Proteobacteria tended to increase or increased significantly, succinic acid vibrios (*Succinivibrio*) and rothia (*Roseburia*) and slaughter bacteria genera (*Blautia*) the relative abundance of the starch decomposition microbes such as add, reduce the relative abundance of cellulose decomposition microbes.

In this study, alpha diversity indices showed that fed grains could change the structure and diversity of bacteria in foal's faecal matter. The sample from barley fed foals significantly increased Shannon and Simpson indexes and altered the community structure of bacteria in foal's faecal matter. The sample from oat fed foals had a significantly increased Chao1 index and changed the diversity of bacteria in the foal's faecal matter. The relative abundance of faecal bacteria results showed that Actinobacteria, Streptococcaceae, Lactobacillaceae, *Streptococcus*, *Lactobacillus* and *Actinobacillus* were significantly increased when corn was fed. The relative abundance of Firmicutes, Rikenellaceae and *Lactobacillus* in

faecal matter was significantly increased when oat was provided. Lachnospiraceae and *Agathobacter* were significantly increased when barley was fed. A study by Defombelle *et al.*(2001) looked at the effect of changing the diet from 100% hay (100:0) to 50% hay and 50% barley. The results showed that concentrations of lactate-utilizing bacteria were not significantly altered in the caecum and colon of ponies. However, the concentrations of lactobacilli and streptococci were significantly increased 5 hours after altering the diet, and then significantly decreased after 29 hours.

Ruminococcaceae bacteria have previously been identified as fibrinolytic bacteria (Daly *et al.*,2012). In this study, the LEfSe result showed that Ruminococcaceae amounts were significantly different in faecal matter from barley fed foals. A study by Bulmer *et al.*(2019) demonstrated that even a small addition of starch to the diet was enough to reduce this bacterial Ruminococcaceae population. Our research showed that the relative abundance of Ruminococcaceae in the corn, oat and barley group was:

Ruminococcus_sp_HUN007 (0.16%,0.13%–0.47%, respectively) and *Ruminococcus flavofaciens* (0.01%, 0.02%, 0.17%, respectively). Research has shown that the extent of the alterations to faecal microbiota with the addition of starch to the diet can be influenced by the source of starch feed (Harlow *et al.*,2016). Our study showed that Lactobacillales, Lactobacillaceae, *Lactobacillus* and *Lactobacillus hayakitensis* (14.19%, 18.09% and 7.11% in faecal matter when fed corn, oat and barley, respectively) were significantly different in the oat fed group compared to the corn and barley fed group. An increase in lactic-acid producing bacteria has also been reported to be coupled with a corresponding decrease in fibrolytic bacterial abundance (Daly *et al.*,2012). The extent of starch digestion in the small intestine can be affected by processing (Potter *et al.*,1992; Meyer *et al.*,1995), so the amount of starch that will result in bypass to the large intestine is likely to vary with both starch source and processing. In this study, the grains were treated by steam pressure, and the processing method was consistent. The reason for the difference in bacterial flora may be related to the structure and proportion of cereal starch depending on the source.

The results of functional prediction showed that long-term feeding of three different grains had varying effects on digestive physiology and health of foals. Results from Bulmer *et al.*(2019) showed that the dietary change resulted in alterations in behaviour and faecal microbiota. The diet could change faecal microbial community composition and relative abundance. Increased starch in the current study had an undesirable effect on behaviour and gut microbiota; it made the ponies more reactive in their behaviour and moved the microbial community composition of the gut towards dysbiosis. However, the opposite was true of the high fibre diet. In this study, we didn't observe the abnormal behaviour and faecal bacteria in foals when fed corn, oat or barley for 60 days. Intestinal microorganisms have specific effects on immune function, nutrient absorption, and even enzyme metabolism (Martin *et al.*,2010). The Tax4Fun prediction was that the diet of corn, oat or barley could affect digestion and metabolism in foals, and the effects of the three grains supplementation on microbial function prediction were different.

Conclusions

In our study, differences were seen in the faecal microbiota of foals fed either corn, oat or barley, and also differences in the overall growth of the foals. Different grains have different impact on faecal microbiota, which are mainly related to the grain sources. Further investigation is required to look at the potential impact of changes in the microbiota on the functional impact on foals when fed grains.

Declarations

Ethics declarations

Ethics approval and consent to participate

All protocols were approved by the Animal Care and Use Committee of Xinjiang Agricultural University (permission number 2018012). All methods were carried out in accordance with relevant guidelines and regulations for the use of animal subjects. The study was carried out in compliance with the ARRIVE guidelines.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Li X B, Yang K L, Zang C J. were involved in study design and execution, data interpretation, and manuscript preparation. Huang X X, Ma C, Chen K X, Zhao G D, Li X Y, Zhang W J, Li Q. performed animals feed, sample collection and data analysis. All authors read and approved the final manuscript.

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Authors' information

Xiaobin Li, male, associate professor, research direction: growth and metabolism of herbivores, College of Animal Science, Xinjiang Agricultural University, China.

Xinxin Huang, male, postgraduate, research direction: Animal Nutrition and Feed Science , College of Animal Science, Xinjiang Agricultural University, China.

Changjiang Zang, male, associate professor, research direction: Animal Nutrition and Feed Science , College of Animal Science, Xinjiang Agricultural University, China.

Chen Ma, male, PhD, research direction: Ruminant Nutrition , College of Animal Science, Xinjiang Agricultural University, China.

Kaixu Chen, male, PhD, research direction: Genetics and Breeding , College of Animal Science, Xinjiang Agricultural University, China.

Guodong Zhao, male, PhD, research direction: Theory and Technology of Herbivore Reproduction , College of Animal Science, Xinjiang Agricultural University, China.

Qian Li, female, postgraduate, research direction: Animal Nutrition and Feed Science , College of Animal Science, Xinjiang Agricultural University, China.

Xuanyue Li, male, postgraduate, research direction: Animal Nutrition and Feed Science , College of Animal Science, Xinjiang Agricultural University, China.

Wenjie Zhang, male, PhD, research direction: Herbivore Nutrition Research , College of Animal Science, Xinjiang Agricultural University, China.

Kailun Yang,, male, professor, research direction: Herbivore Nutrition and Metabolism , College of Animal Science, Xinjiang Agricultural University, China.

Informed consent

Owners gave informed consent for their animals'inclusion in the study.

References

1. Marcio,C.C.,Henry,R.S.,Luis,G.A.,Emma,Allen-Vercoe.,Roberta,G.G.Weese,J.S. Changes in the equine fecal microbiota associated with the use of systemic antimicrobial drugs. *BMC Veterinary Research*.11(1),19(2015)
2. Glinsky,M.J.,Smith,R.M.,Spires,H.R.and Davis,C.L. Measurement of volatile fatty acid production rates in the cecum of the pony. *Journal Anim Science*.42(6),1465-70(1976)
3. Daly,K.,Proudman,C.J.,Duncan,S.H.,Flint,H.J.,Dyer,J.,Shirazi-Beechey,S.P. Alterations in microbiota and fermentation products in equine large intestine in response to dietary variation and intestinal

- disease. *British Journal Nutrition*.107(7),989-95(2012)
4. Costa,M.C.,Arroyo,L.G.,Allen-Vercoe,E.,Stämpfli,H.R.,Kim,P.T.,Sturgeon,A.,Weese,J.S.Comparison of the fecal microbiota of healthy horses and horses with colitis by high throughput sequencing of the V3-V5 region of the 16S rRNA gene. *Plos one*.7(7),e41484(2012).
 5. Svihus,B.,Uhlen,A.K.,Harstad,O.M. Effect of starch granule structure,associated components and processing on nutritive value of cereal starch: A review. *Animal Feed Science and Technology*.122(3),303-320(2005)
 6. Tester,R.F.,Qi,X.,Karkalas,J. Hydrolysis of native starches with amylases. *Anim Feed Science and Technology*.130(1),39-54(2006)
 7. Defombelle,A.,Julliand,V.,Drogoul,C.,Jacotot,E., Feeding and microbial disorders in horses:1-Effects of an abrupt incorporation of two levels of barley in a hay diet on microbial profile and activities. *Journal of Equine Veterinary Science*.21(9),439-445(2001)
 8. Medina,B.,Girard,I.D.,Jacotot,E.,Julliand,V. Effect of a preparation of *Saccharomyces cerevisiae* on microbial profiles and fermentation patterns in the large intestine of horses fed a high fiber or a high starch diet. *Journal Anim Science*.80(10),2600-9(2002)
 9. Willing,B.,Vörös,A.,Roos,S.,Jones,C.,Jansson,A.,Lindberg,J.E. Changes in faecal bacteria associated with concentrate and forage-only diets fed to horses in training. *Equine Veterinary Journal*.41(9),908-14(2009)
 10. Kienzle,E.,Pohlenz,J.,Radicke,S. Morphology of starch digestion in the horse. *Transboundary and Emerging Diseases*.44(4),207-21(1997)
 11. Rowe,J.B.,Choct,M.,Pethick,D.W.(1999).Processing cereal grains for animal feeding. *Australian Journal of Agricultural Research*.50,721-736.
 12. Magoč,T.,Salzberg,S.L. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*.27(21),2957-2963(2011)
 13. Caporaso,J.G.,Kuczynski,J.,Stombaugh,J.,Bittinger,K.,Bushman,F.D.,Costello,E.K., et al. QIIME allows analysis of high-throughput community sequencing data. *Nature methods*.7(5),335-336(2010)
 14. Wang,Q.,Garrity,G.M.,Tiedje,J.M.,Cole,J.R. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and environmental microbiology*.73(16),5261-7(2007)
 15. McDonald,D.,Price,M.N.,Goodrich,J.,Nawrocki,E.P.,DeSantis,T.Z.,Probst,A., et al. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *The ISME journal*.6(3),610-618(2012)
 16. Rideout,J.R.,He,Y.,Navas-Molina,J.A.,Walters,W.A.,Ursell,L.K.,Gibbons,S.M., et al. Subsampled open-reference clustering creates consistent, comprehensive OTU definitions and scales to billions of sequences. *PeerJ*.2:e554(2014)
 17. Cole,J.R.,Wang,Q.,Cardenas,E.,Fish,J.,Chai.B.,Farris,R.J., et al. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic acids research*.37(Database issue),141-145(2009)

18. Langille,M.G.I.,Zaneveld,J.,Caporaso,J.G.,McDonald,D.,Knights.D.,Reyes.J.A., et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology*.31(9),814-821(2013)
19. Li,B.,Zhang,X.X.,Guo,F.,Wu,W.M.,Zhang,T. Characterization of tetracycline resistant bacterial community in saline activated sludge using batch stress incubation with high-throughput sequencing analysis. *Water research*.47(13),4207-4216(2013)
20. Segata,N.,Lzard,J.,Waldron,L.,Gevers,D.,Miropolsky,L.,Garrett,W.S.,Huttenhower,C. Metagenomic biomarker discovery and explanation. *Genome Biology*.12(6),R60(2011)
21. Aßhauer,K.P.,Wemheuer,B.,Daniel,R.,Meinicke,P. Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data. *Bioinformatics*.31(17),2882-2884(2015)
22. Doblado Maldonado,A.F.,Gomand,S.V.,Goderis,B.,Delcour,J.A. Methodologies for producing amylose: A review. *Critical Reviews in Food Science and Nutrition*.57(2),407-417(2017)
23. Tester,R.F.,Karkalas,J.,Qi,X., Starch-composition, fine structure and architecture. *Journal of Cereal Science*.39(2),151-165(2004)
24. Gómez,L.M.,Posada,S.L.,Ángel,O.M. Starch in ruminant diets:a review. *Revista Colombiana de Ciencias Pecuarias*.29(2),77-90(2016)
25. Pérez,S.,and Bertoft,E. The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. *Starch-Stärke*.62(8),389-420(2010)
26. Meyer,H.,Radicke,S.,Kienzle,E.,Wilke,S.,Kleffken,D. Investigations on preileal digestion of oats, corn and barley starch in relation to grain processing. *Equine Nutrition and physiology symposium*.92-97(1993)
27. Mohsen,R.,Mehdi,K&B.,Mehdi,H.Y,Mehdi,M. Effects of corn grain processing method (ground versus steam-flaked) with rumen undegradable to degradable protein ratio on growth performance, ruminal fermentation, and microbial protein yield in Holstein dairy calves. *Animal Feed Science and Technology*.269,114646(2020)
28. Potter,G.D.,Arnold,F.F.,Householder,D.D.,Hansen,D.H.,Brown,K.M. Digestion of starch in the small or large intestine of the equine. *Pferdeheilkunde*.1(4),107-111(1992)
29. Meyer,H.,Radicke,S.,Kienzle,E.,Wilke,S.,Kleffken,D.,Illenseer,M. Investigations of preileal digestion of starch from grain, potato and manioc in horses. *Transboundary and Emerging Diseases*.42(6),371-81(1995)
30. Kienzle,E.,Radicke,S.,Wilke,S.,Landes,E.,Meyer,H. Preileal starch digestion in relation to source and preparation of starch. *In: Pferdeheilkunde (1st European Conference on Horse Nutrition)*.103(1992)
31. Weurding,R.E.,Veldman,A.,Veen,W.A.,Vanderaar,P.J.,Verstegen,M.W. Starch digestion rate in the small intestine of broiler chickens differs among feedstuffs. *The Journal of Nutrition*.131(9),2329-2335(2001)
32. Nasir,Z.,Wang,L.F.,Young,M.G.,Swift,M.L.,Beltranena,E.,Zijlstra,R.T. The effect of feeding barley on diet nutrient digestibility and growth performance of starter pigs. *Animal Feed Science and Technology*.210,287-294(2015)

33. Kong,B.W.,Kim,J.I.,Kim,M.J.,Kim,J.C. Porcine Pancreatic α -Amylase Hydrolysis of Native Starch Granules as a Function of Granule Surface Area. *Biotechnology Progress*.19(4),1162-1166(2003)
34. Defombelle,A.,Veiga,L.,Drogoul,C.,Julliand,V. Effect of diet composition and feeding pattern on the prececal digestibility of starches from diverse botanical origins measured with the mobile nylon bag technique in horses. *Journal of Animal Science*.82(12),3625-3634(2004)
35. Kienzle,E.,Radicke,S.,Landes,E.,kleffken,D.,Illenseer,M.,Meyer,H. Activity of amylase in the gastrointestinal tract of the horse. *Journal of animal physiology and animal nutrition*.72,234-241(1994)
36. Black,J.L. A theoretical consideration of the effect of preventing rumen fermentation on the efficiency of utilisation of dietary energy and protein in lambs. *British journal of nutrition*.25,31-55(1971)
37. Garner,H.E.,Moore,J.N.,Johnson,J.H.,Clark,L.,Amend,J.,Tritschler,L.G.,et al. Changes in the Caecal Flora associated with the Onset of Laminitis. *Equine Veterinary Journal*.10(4),249-252(1978)
38. Harlow,B.E.,Lawrence,L.M.,Hayes,S.H.,Crum,A.,Flythe,M.D. Effect of dietary starch source and concentration on equine fecal microbiota. *The Public Library of Science one*.11(4),e0154037(2016)
39. Rosenfeld,I.,and Austbø,D. Digestion of cereals in the equine gastrointestinal tract measured by the mobile bag technique on caecally cannulated horses. *Animal Feed Science and Technology*.150(3),249-258(2008)
40. Harlow,B.E.,Donley,T.M.,Lawrence,L.M.,Flythe,M.D. Effect of starch source (corn, oats or wheat) and concentration on fermentation by equine faecal microbiota in vitro. *Journal of Applied Microbiology*.119(5),1234-1244(2015)
41. Radicke,S.,Kienzle,E.,Meyer,H. Preileal apparent digestibility of oats and corn starch and consequences forcecal metabolism. *Proceedings of the 12th Equine Nutrition Physiology Symposium, Calgary*.(1),43-48(1991)
42. Ren,H.,Su,X.D.,Bai,H.X.,Yang,Y.T.,Wang,H.R.,Dan,Z.,et al. Specific enrichment of microbes and increased ruminal propionate production: the potential mechanism underlying the high energy efficiency of Holstein heifers fed steam-flaked corn. *AMB Express*.9(1),209(2019)
43. Bulmer,L.S.,Murray,J.A.,Burns,N.M.,Garber,A.,Wemelsfelder,F.,McEwan,N.R.,Hastie,P.M.High-starch diets alter equine faecal microbiota and increase behavioural reactivity. *Scientific Reports*.9(1),701-712(2019)
44. Martin,R.,Nauta,A.J.,Benamor,K.,Knippels,L.M.J,Knol,J.,Garssen,J. Early life:Gut microbiota and immune development in infancy. *Beneficial Microbes*.1(4),367-382(2010)

Figures

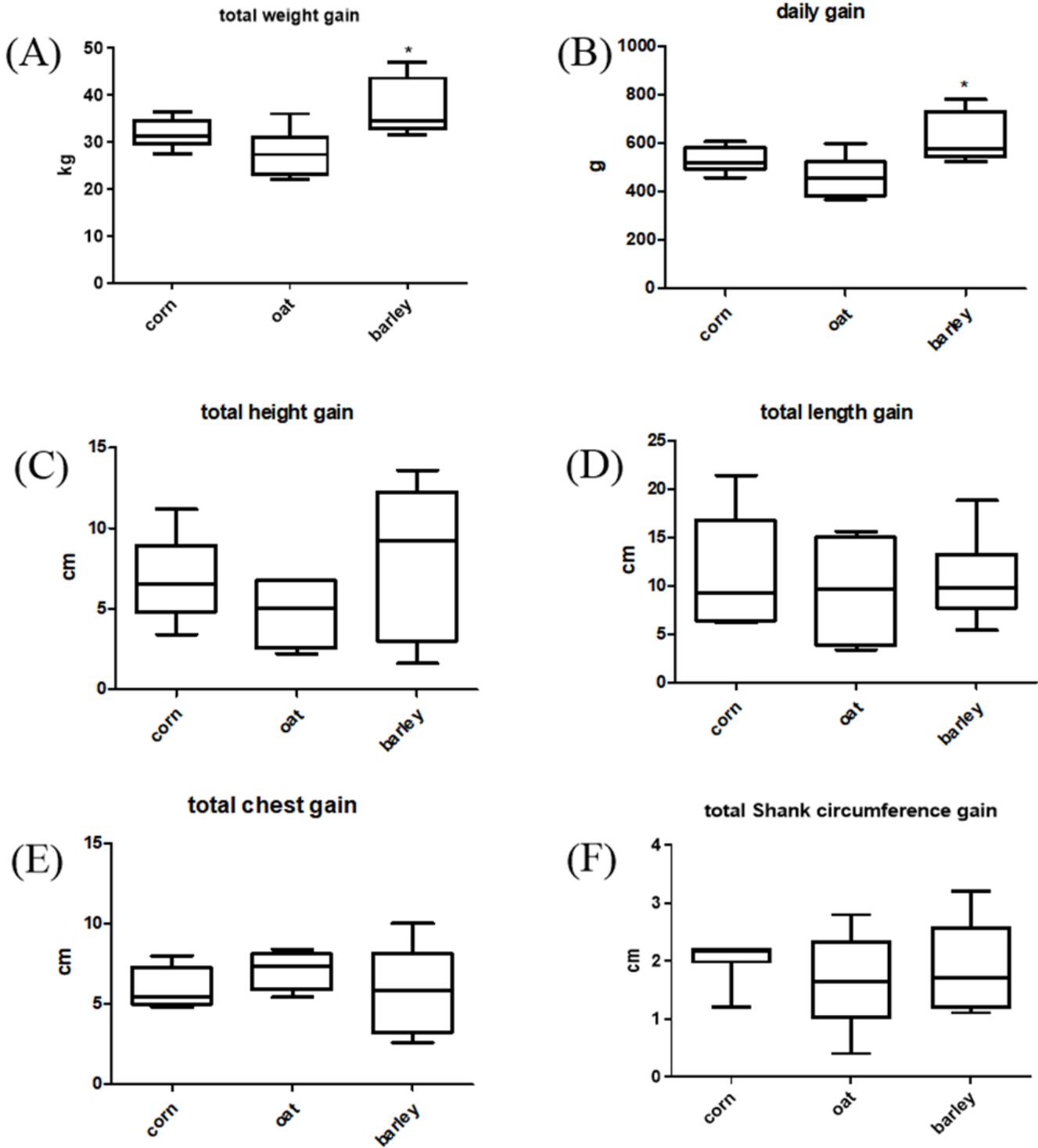


Figure 1

The effect of the source of grains on the total and average daily increases of body weight and body size of the foals. (A) Box and whisker plots showing the total weight gain of foals from different grain sources during the trial period. (B) A box-and-whisker plot showing the effect of different grain sources on average daily gain of foals during the trial period. (C) A box-and-whisker plot showing the effect of different grain sources on the total body height of the foal during the trial period. (D) A box-and-whisker plot showing the

effect of different grain sources on the total increase in foal body length during the trial period.(E) Box and whisker plots showing the effect of different grain sources on the total chest circumference of the foal during the trial period.(F) Box and whisker plots showing the increase in the total tubules circumference of foals from different grain sources during the trial period.

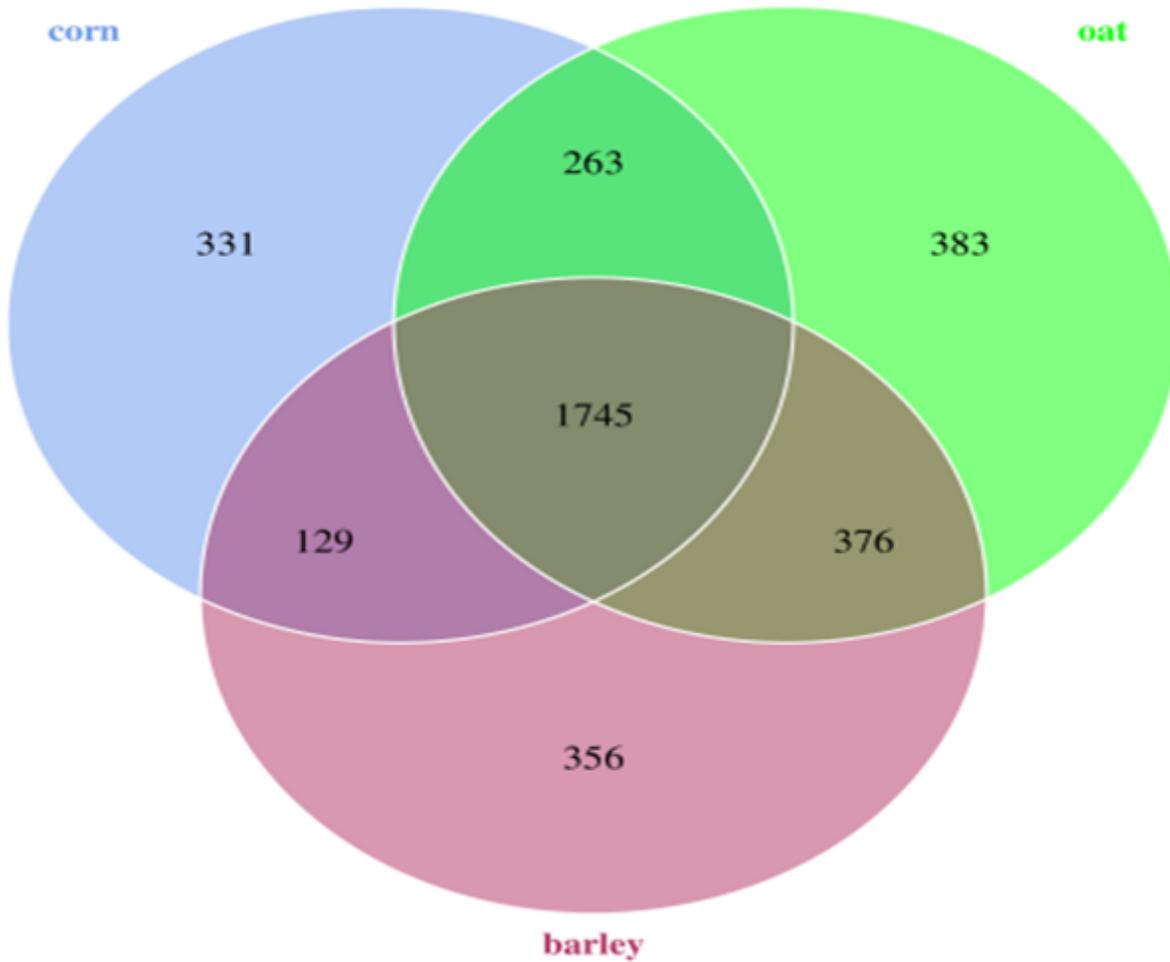


Figure 2

Venn graph based on OTU.Each circle in the figure represents a group of samples; the number of circles and the overlap of circles represents the number of common OTUs among all experimental groups; the number of unique OTUs among all experimental groups without overlap represents the number of unique OTUs among all experimental groups.

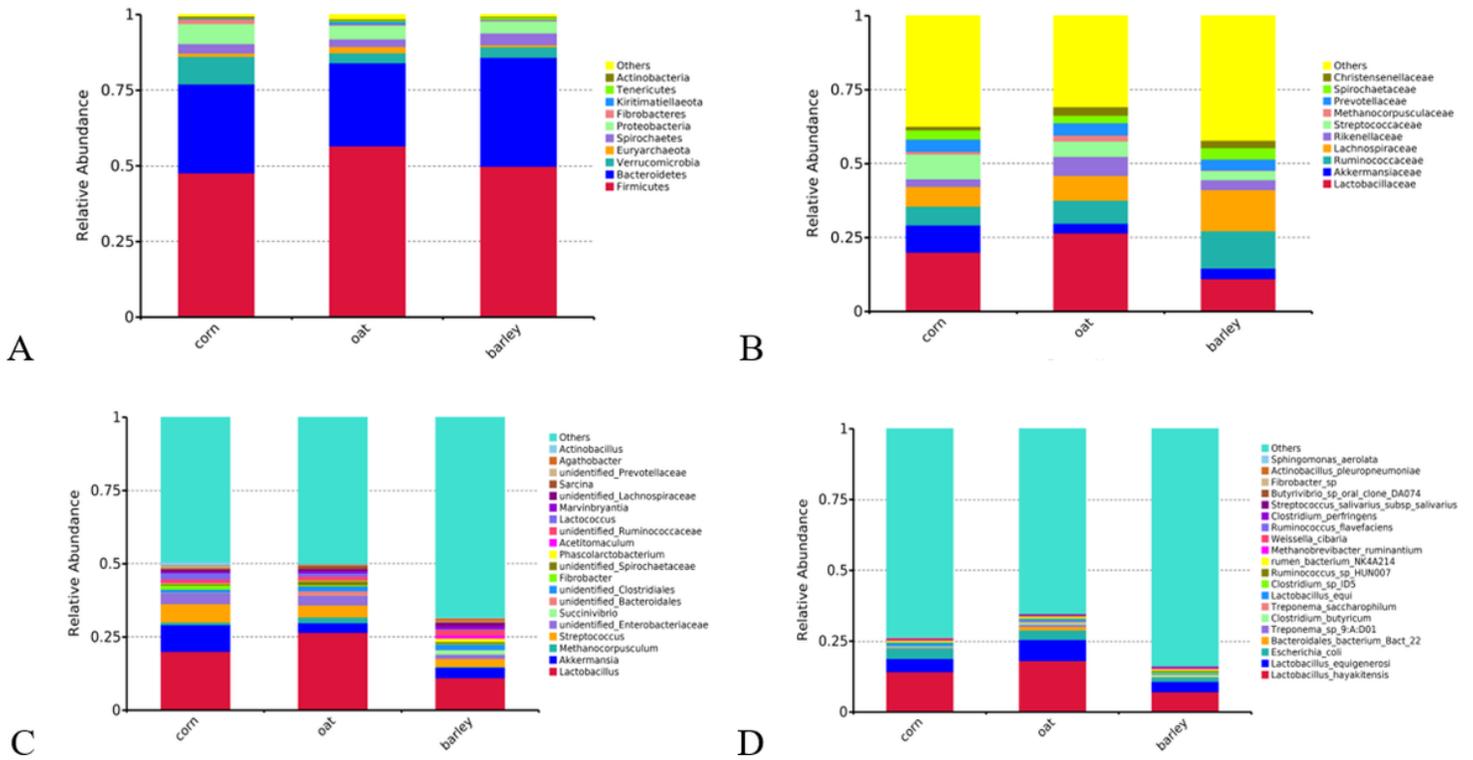


Figure 3

Relative abundance of faecal bacteria at the phylum (A), family (B), genus (C) and species (D) levels. (A) histogram of the relative abundances of the first 10 phylum levels of bacteria in foal feces, where "Other" refers to the sum of the relative abundances of all species at the eleventh and subsequent levels of the taxonomic level. (B) histogram of relative abundance of horizontal bacteria of the first 10 families in foal feces. (C) histogram of relative abundance of horizontal bacteria of the first 20 genera in foal feces. (D) histogram of the relative abundances of the first 20 levels of bacteria in foal feces.

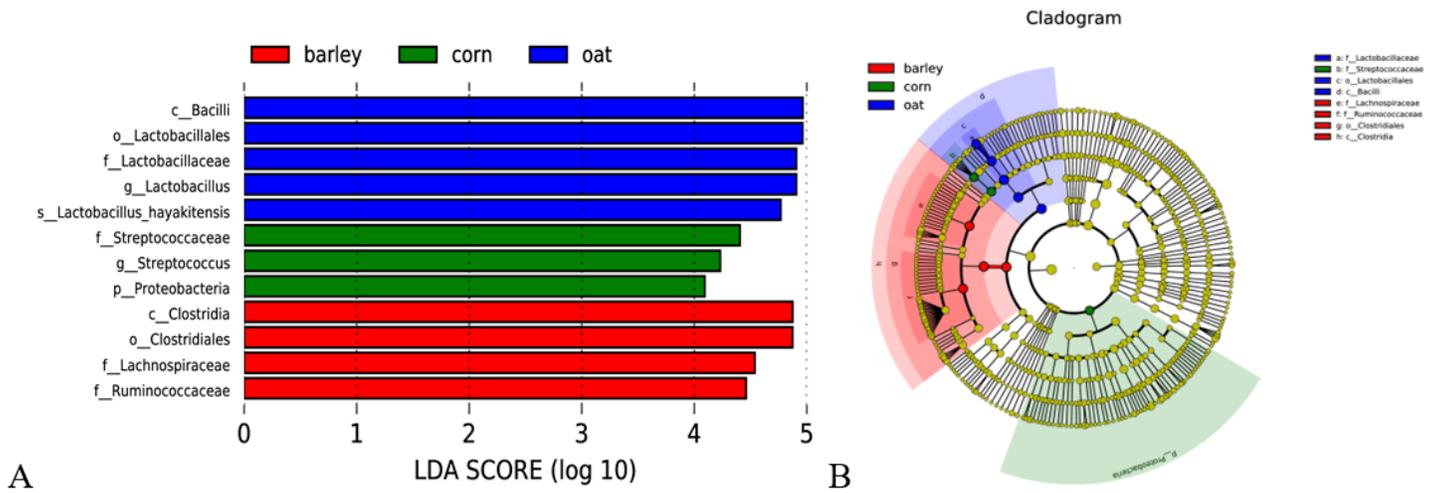


Figure 4

LDA Effect Size of statistically different biomarkers in fecal. Non-parametric factorial Kruskal-Wallis (KW) Sum-Ranktest (non-parametric Kruskal-Wallis and ranktest) was used to detect the species with significant difference in bacterial abundance in the feces of foals from different grain sources, and then the difference between groups was determined by group Wilcoxon rank sum test. Finally, linear discriminant analysis (LDA) was used to reduce and evaluate the impact size of significantly different species (i.e., LDA Score). (A) Histogram of LDA value distribution, showing the biomarkers with statistical differences between groups with LDA Score greater than the set value of 4. The length of the bar chart represents the influence size of different species (i.e., LDA Score). The blue is the species with significant abundance difference in the oat group, the green is the species with significant abundance difference in the maize group, and the red barley group. (B) Evolutionary branching diagram under LEFSE analysis. In the figure, the circles radiating from the inside to the outside represent taxonomic levels from phylum to genus (or species). Each small circle at different taxonomic levels represents a taxon at that level, and the diameter of the small circle is proportional to the relative abundance. Coloring principle: no significant difference of species uniform color is yellow, differences between species Biomarker follow group staining, red nodes play an important role in the barley group of microbial groups, said green nodes play an important role in corn group of microbial groups, said the blue nodes play an important role in the oat group of microbial groups.)

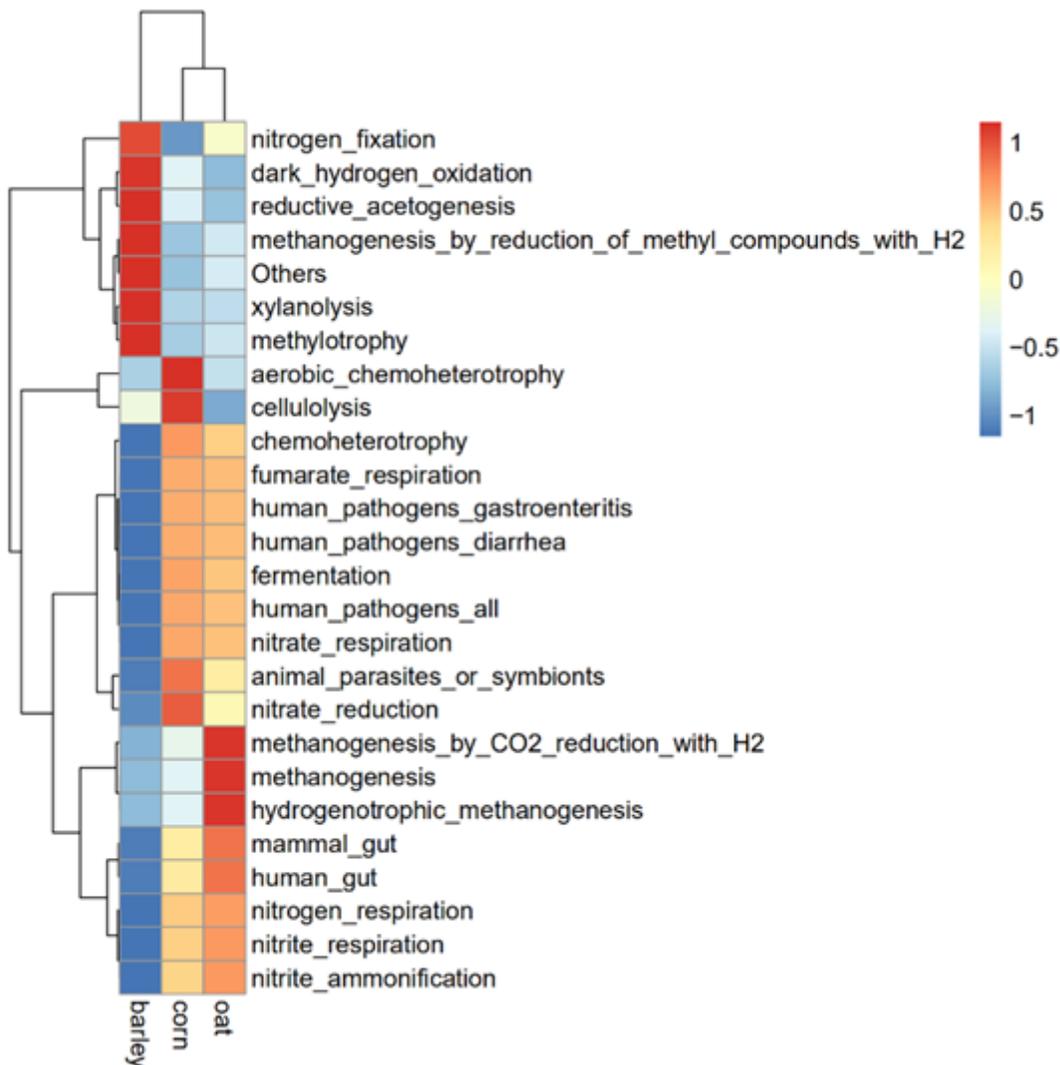


Figure 5

Tax4Fun function annotation clustering heat map. According to the functional annotation and abundance information of the samples in the data, the top 25 functions in the list of abundance and the abundance information of each group of samples were selected to draw a heat map, and the clustering was conducted from the level of functional differences. Functional notes are on the right side of the heat map, and test groups are on the lower side of the heat map. In the heat map, the red color represents the higher relative abundance of functions, and the relative abundance increases with the deepening of the color. Blue represents the higher the relative abundance of the function, and with the deepening of the color, the relative abundance decreases.

Supplementary Files

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- [Table5originaldata.xls](#)