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Starter feed enhances growth, antioxidant capacity, and gut microbiota in Chawula yak calves

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Yak has ability to adapt the harsh environments like extremely cold, anoxic, strong ultraviolet rays and shortage of pasture. It is observed that the nutritional supply during the preweaning period is highly associated with the development of the gastrointestinal tract and immunity of animals. In this study, twenty-one male yak calves were divided into control group (DFC), starter feed group 1 (DFO), and starter feed group 2 (DFT) to study the growth of early weaning Chawula yak calves. Calves in group DFC were free-ranged, DFO group were fed with alfalfa (1.4 kg/head/day) and starter feed 1 (1.4 kg/head/day), and DFT group were fed with alfalfa (1.4 kg/head/day) and starter feed 2 (1.4 kg/head/day) for 6 weeks. The body weight of yak calves in DFT was significantly higher than that of control yaks in DFC ($P < 0.05$), and the net weight growth rate in DFO ($P < 0.001$) and DFT ($P < 0.0001$) were both obviously higher than that in the DFC group. The chest girth (bust) in group DFO ($P < 0.05$) and DFT ($P < 0.05$) were both markedly higher than that in the DFC group. The serum contents of T-AOC in DFC calves were markedly lower than DFO ($P < 0.01$) and DFT ($P < 0.001$) yaks. Also, T-AOC was obviously higher in DFT yaks than in DFO animals ($P < 0.05$). The levels of GSH-Px were significantly higher in DFO ($P < 0.01$) and DFT ($P < 0.01$) yaks than DFC yaks. High throughout sequencing achieved 391520, 356907 and 353763 filtered sequences in DFC, DFO and DFT yaks, and one phylum (Firmicutes B 370539) and thirty-seven genera (*Phocaeicola* A 858004, *Cryptobacteroides*, *Evtepia*, CAG-273, etc.) were identified as biomarkers in yak calves. We observed that starter feeds could promote the growth of early weaning Chawula yak calves by enhancing antioxidant capacity and regulating the gut microbiota.

Keywords Yak, Starter feeds, Oxidation resistance, Microbiota, Sequencing

Yak is an ancient even-toed ruminant animal residing in the Qinghai-xizang plateau and its surrounding regions with altitude over 3000 m[1,2]. As a results long time natural domestication, those herbivores own ability to adapted the harsh environments like extremely cold, anoxic, strong ultraviolet rays and shortage of pasture[3]. There are around 14–17 million yaks globally including countries of China, Nepal, Bhutan, India and others, and 90%of them are raised in China[4]. Yaks can provide nutritional milk and meat, high-quality fur, fermented manure, and low-cost fuel, and serve as a means of transport, which makes them critically important to local people in the cold plateau regions[5]. Chawula yak is mainly distributed in the Nyainrong County, Naqu, China, with a northern latitude of 32° 06′ and an east longitude of 92° 18′. Nyainrong County has an average altitude of more than 4700 m, with an annual average precipitation of 400 mm and a temperature of around 0 °C. Hence, highly efficient breeding of yaks is of great importance to local economic and farmer livings.

It is common sense that the nutritional supply of calves during the preweaning period is highly associated with the development of the gastrointestinal tract and immunity of animals[6]. The starter feed supplementation in yak calves significantly improves growth performance, promotes rumen and organ development, and alters the gut microbiota, thus starter feeds are generally more effective than traditional maternal grazing for productivity in barn-fed conditions. Therefore, products like milk replacer, starter, and alfalfa hay were produced to promote the growth of calves[7]. Calf starter feeds are composed of delicious and digestible ingredients, which are commonly employed for the transition of young ruminants from breast feeding to solid feeds[8]. The complete microbial

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community, namely microbiota, consists of trillions of microbes, including viruses, prokaryotes, and eukaryotes[9], which is connected with the hosts' digestive absorption, metabolism, intestinal barrier, and immunity[10,11]. The gut microbiota is dynamic (amount, vitality and composition) affected by factors of age, genetic variation, diet, lifestyle, medications and others[12], which are related to the development and treatment of many diseases[13]. In the reorganization of the important functions of microbiota, more and more attentions were paid to the regulation of microbiota for finding novel therapies like faecal microbiota transplantation, specific additives, and probiotics[14–16]. Previous studies found that starter feeds promoted lambs, yaks and piglets' growth via alliterating microbiota[7,17,18]. However, there is limited research about the effect of starter feeds on Chawula yak calves. Therefore, we carried out this experiment to disclose the impact of starter feeds on the growth, antioxidant ability and intestinal microbiota of early weaning Chawula yak calves to explore novel highly efficient breeding methods in plateau regions.

Results

Weight, body sizes and antioxidant ability of yak calves

The body weight of yak calves in DFT was signally higher than that of control animals in DFC ($P<0.05$), and the net weight growth rate in DFO ($P<0.001$) and DFT ($P<0.0001$) were both obviously higher than that in the DFC group (Fig. 1a). DFT calves gained 4 kg more than control (DFC) group. The chest girth (bust) in group DFO ($P<0.05$) and DFT ($P<0.05$) were both markedly higher than that in the DFC group, where there was no observable difference of height, body length, body height, and circumference of cannon bone among calves in different groups (Fig. 1b). The serum contents of T-AOC in DFC calves were markedly lower than DFO ($P<0.01$) and DFT ($P<0.001$) yaks. Also, T-AOC was obviously higher in DFT yaks than in DFO animals ($P<0.05$). The levels of GSH-Px were significantly higher in DFO ($P<0.01$) and DFT ($P<0.01$) yaks than DFC yaks. In contrast, no obvious difference was detected in SOD and MDA levels among yak calves in different groups (Fig. 1c).

Sequencing data of yak calves in different groups

There were more than 47,000 (DFC > 55,700, DFO > 48,800, DFT > 47,500) raw and 45,000 (DFC > 51,500, DFO > 44,600, DFT > 45,000) filtered reads in yak calf (Table 1). There were 7396 ASVs in yak calves, with 193 shared ASVs in the three groups (Fig. 2a). There was no significant difference of the α-diversity index among different yak groups (Table 2, Fig. 2b). The rarefaction curves of all yak calves, indicating that those samples were sufficient to represent the bacterial diversity (Fig. 2c). All of the yaks' rank abundance curves were gently in a horizontal direction, reflecting higher evenness in yaks (Fig. 2d).

Microbiota comparison analysis of yak calves in different taxonomic levels

In the phylum level, Bacteroidota and Firmicutes A were the primary phyla in DFC (66.10%, 23.84%), DFO (68.25%, 29.07%), and DFT (66.81%, 29.53%) (Fig. 3a). At the class level, Bacteroidia and Clostridia 258483

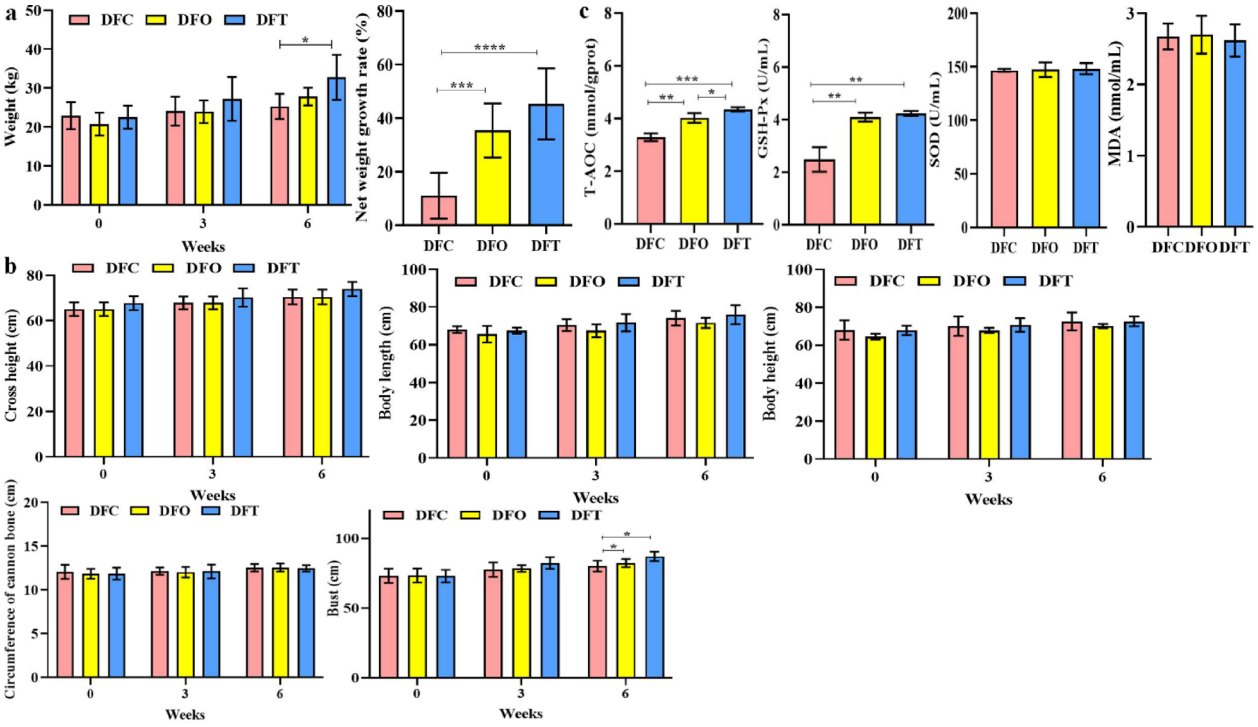

Fig. 1. Weight, body sizes and antioxidant ability compared analysis of yak calves in different groups. (**a**) Body weight, (**b**) body size, (**c**) Antioxidant ability. Data are presented as SD, * $P<0.05$, ** $P<0.01$, *** $P<0.001$, and *** $P<0.0001$.

Samples	Input	Filtered	Denoised	Merged	Non-chimeric	Non-singleton
DFC1	79,526	74,749	73,027	62,923	58,866	58,837
DFC2	55,728	51,509	50,130	43,761	40,022	39,998
DFC3	79,626	74,461	73,108	64,663	60,739	60,726
DFC4	61,066	56,153	55,456	51,827	51,524	51,521
DFC5	66,012	60,867	59,296	52,157	50,559	50,518
DFC6	79,329	73,781	73,324	71,198	68,619	68,612
DFO1	58,807	53,429	51,862	42,739	40,280	40,253
DFO2	79,388	74,336	71,850	53,886	49,576	49,511
DFO3	48,826	44,655	43,553	38,154	36,317	36,294
DFO4	60,229	54,457	53,220	46,978	44,421	44,391
DFO5	60,434	55,428	53,503	42,586	39,854	39,815
DFO6	79,518	74,602	72,430	49,029	42,774	42,674
DFT1	74,538	68,122	65,983	54,339	49,911	49,849
DFT2	47,593	43,330	41,601	33,822	30,798	30,752
DFT3	79,243	74,003	73,413	70,163	65,100	65,089
DFT4	49,363	45,098	43,471	34,734	32,612	32,573
DFT5	79,483	74,813	72,238	56,466	51,423	51,353
DFT6	52,748	48,397	46,313	37,443	34,546	34,483

Table 1. Statistical analysis of sequencing data of calves.

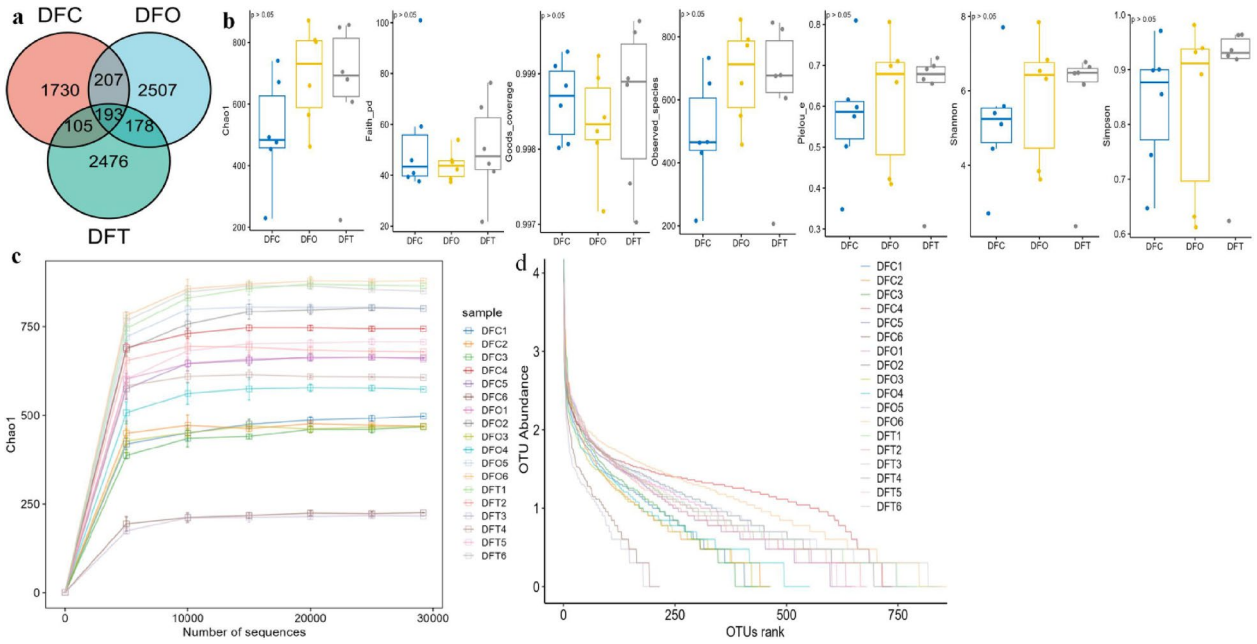

Fig. 2. Venn map and alpha diversity analysis of calves in different groups. (**a**) Venn map, (**b**) Alpha diversity indexes, (**c**) Rarefaction curve, (**d**) Rank abundance curve.

were the dominating classes in DFC (66.09%, 23.83%), DFO (68.25%, 29.06%) and DFT (66.82%, 29.52%) (Fig. 3b). At the order level, Bacteroidales and Oscillospirales were the main orders in DFC (66.35%, 9.52%) and DFO (69.84%, 13.43%), while Bacteroidales (52.39%) and Chitinophagales (15.90%) were the staple orders in DFT (Fig. 3c). At the family level, Bacteroidaceae and Muribaculaceae were the primary families in DFC (48.68%, 13.53%) and DFO (50.30%, 9.45%), while Bacteroidaceae (36.95%) and Saprospiraceae (17.23%) were the prominent families in DFT (Fig. 3d). At the genus level, the prime in different yak groups were *Paraprevotella* (47.27%) and CAG-485 (13.00) in DFC, Paraprevotella (42.25%) and Phocaeicola A 858004 (9.97%) in DFO, and Paraprevotella (26.41%) and OLB9 (20.63%) in DFT, respectively (Fig. 3e).

Sample	Chao1	Faith pd	Goods coverage	Observed species	Pielou e	Shannon	Simpson
DFC1	492.84121	45.93436481	0.998018074	463.9	0.502029341	4.446751909	0.744094529
DFC2	474.0093415	37.60829445	0.998839597	465.8	0.575099176	5.097414323	0.900056347
DFC3	452.2671701	40.87152561	0.998576025	431.6	0.616180012	5.393750525	0.898558365
DFC4	739.9449634	100.924826	0.999291436	732.8	0.809527113	7.70448255	0.970822455
DFC5	670.7595292	59.15957233	0.998065996	652	0.597475165	5.585626652	0.854968211
DFC6	227.9723873	39.36657463	0.999099747	215.9	0.348034567	2.698711146	0.646610677
DFO1	659.358754	45.88250464	0.998942288	652.6	0.698631121	6.532231866	0.931792115
DFO2	806.8611569	42.32954645	0.997172588	772.7	0.658816237	6.320514335	0.89133182
DFO3	461.684044	45.1332652	0.999236667	457.8	0.409815347	3.622179382	0.612195858
DFO4	564.7831405	37.38309178	0.998237147	549.6	0.422040591	3.841498662	0.631575291
DFO5	802.1721757	53.91975107	0.998421989	791	0.710089238	6.83640612	0.939374505
DFO6	872.4087973	38.64076135	0.998086534	854.7	0.806228076	7.852066648	0.981868316
DFT1	855.2011576	76.37999741	0.997025399	823.7	0.698357608	6.764261054	0.962926497
DFT2	678.9533445	44.56148965	0.999548162	677.9	0.655136564	6.161512024	0.918564812
DFT3	222.4126509	21.74797384	0.998935442	206.2	0.306803438	2.358575042	0.623626805
DFT4	606.185676	41.51383432	0.999698775	605.3	0.718009555	6.635488715	0.963794387
DFT5	706.0121463	50.38999535	0.997545697	675.9	0.690961406	6.495482195	0.935855469
DFT6	849.2889858	66.71523536	0.99885329	844.8	0.666075383	6.475892802	0.9253224

Table 2. Statistical analysis of alpha diversity index in yak calves in different groups.

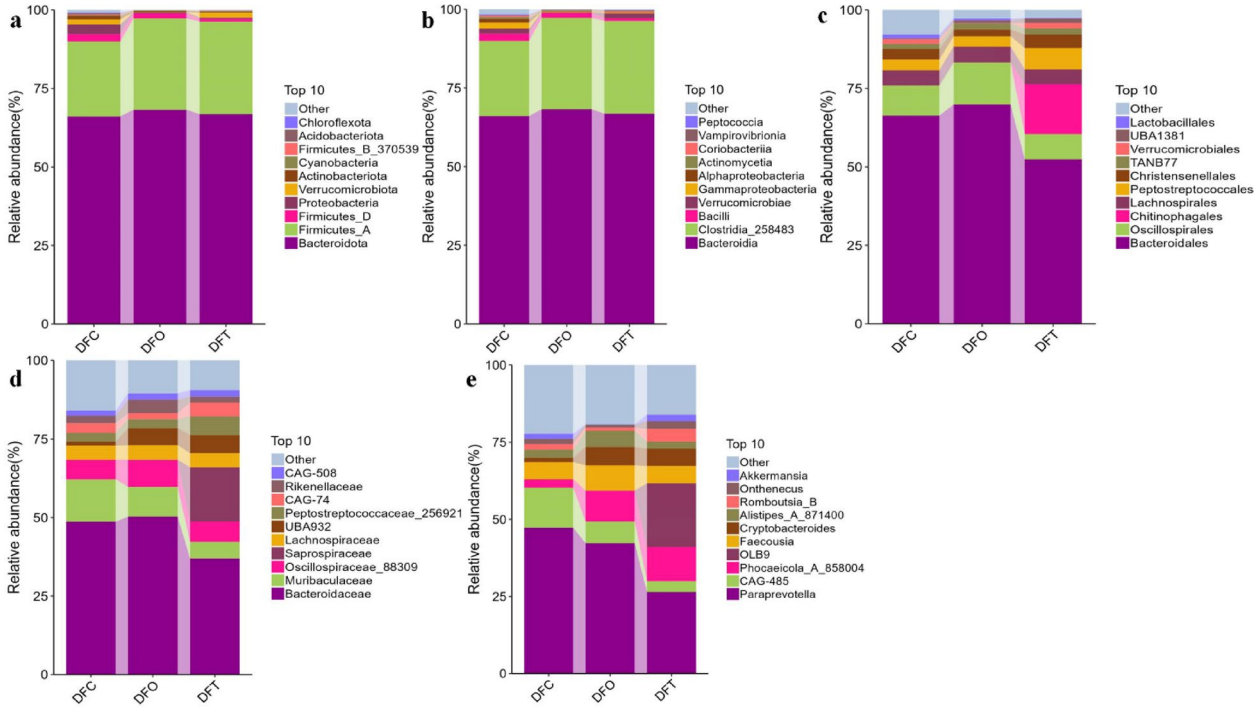

Fig. 3. Microbiota comparing analysis of yak calves in different taxa. (**a**) Phylum, (**b**) Class, (**c**) Order, (**d**) Family, (**e**) Genus.

Exploring biomarkers in yak calves in different groups

B-diversity analysis showed that there a clear distance among the three yak calf groups via PCoA, NMDS, and UPGMA analysis (Fig. 4a–c), and PERMANOVA also confirmed it with an obvious difference ($P = 0.002 < 0.01$, Fig. 4d).

Heatmap showed that phyla of Firmicutes D, Firmicutes B 370539, Patescibacteria, Campylobacterota, Planctomycetota, Actinobacteriota, Gemmatimonadota, Chloroflexota, Desulfobacterota I, Nitrospirota A 437815, Desulfobacterota G 459546, Fusobacteriota, Bdellovibrionota E, Dormibacterota, Desulfobacterota E, Thermoproteota, Methanobacteriota A 1229, Eisenbacteria, Fibrobacterota, SAR324, Myxococcota A 473307, Proteobacteria, Acidobacteriota and Eremiobacterota were higher in DFC yak, and Spirochaetota, Bacteroidota,

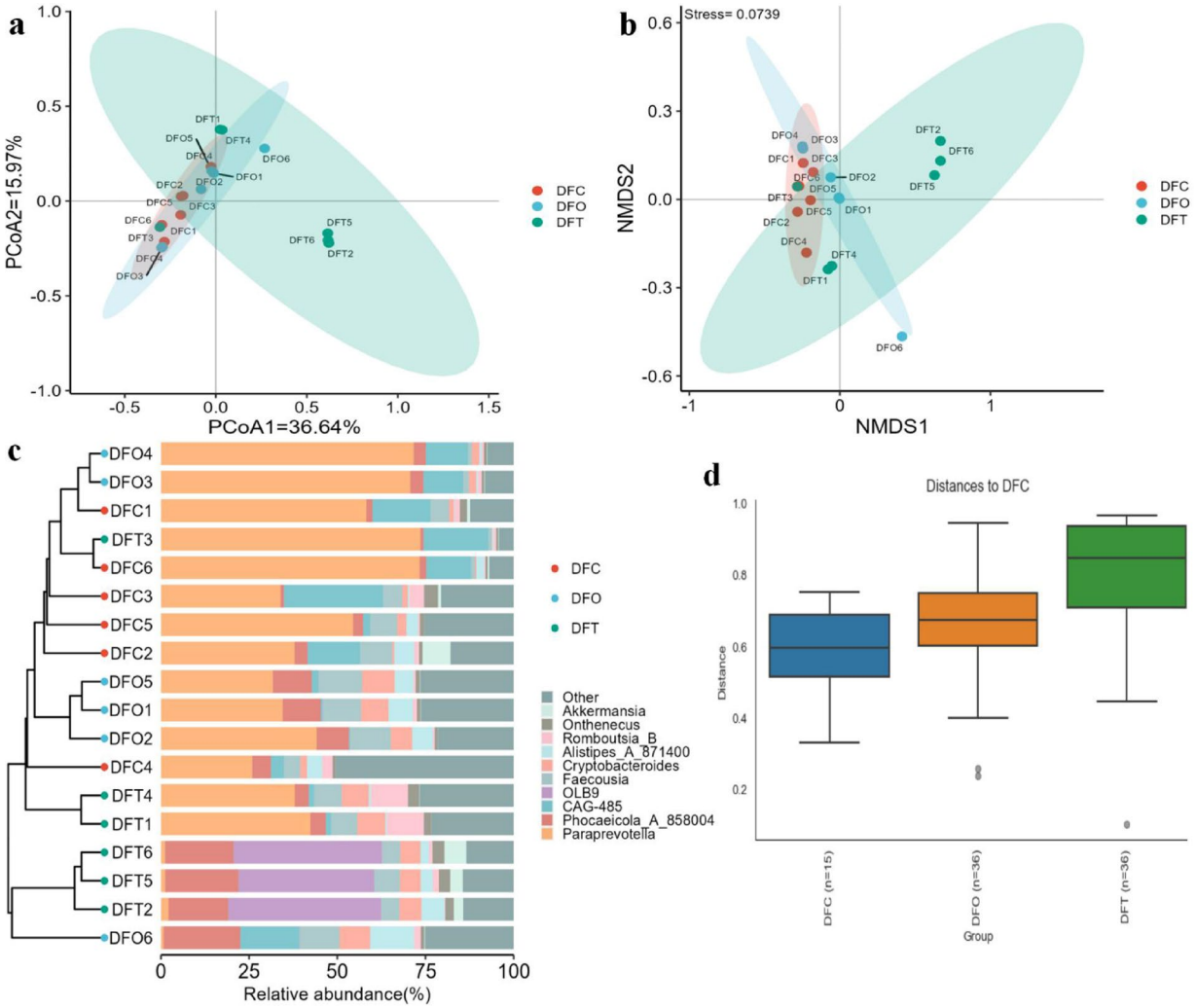

Fig. 4. β-diversity analysis of yak calves in different groups. (**a**) PCoA, (**b**) NMDS, (**c**) UPGMA, (**d**) PERMANOVA.

Firmicutes G and CSP1-3 were higher in DFO ruminants, while Synergistota, Chlamydiota, Elusimicrobiota, Halobacteriota, Marinisomatota and Cyanobacteria were higher in DFT animals (Fig. 5a). At the genus level, the abundance of *Bacteroides H, Limosilactobacillus*, *Peptococcus*, UBA6857, *Acinetobacter*, *Brevundimonas*, *Faecalimonas*, RUG420, *Pygmaiobacter*, *Lactobacillus*, CAG-488, CAG-485, CAG-269, *Enterenecus,* and *Anaerobutyricum* were higher in DFC groups. Jeotgalibaca, *Corynebacterium*, *Paramuribaculum*, RUG13077, *Limivicinus*, Bact-08, *Alistipes* A 871400, UBA4334, UBA737, *Avispirillum*, *Faecousia* and UBA5905 were higher in DFO group, while CCUG-7971, UMGS1994, CAG-273, OLB9, *Turicibacter*, *Onthenecus*, *Limiplasma*, *Fimenecus*, *Copromorpha*, *Romboutsia* B and SFMI01 were higher in the DFT group (Fig. 5b).

LEfSe showed that CAG 485 ($P<0.05$), *Acinetobacter* ($P<0.05$), *Alloprevotella* ($P<0.05$) and CAG 485 ($P<0.05$) were signally higher in DFC groups. *Alistipes* A 871400 ($P<0.01$), *Alloprevotella* ($P<0.01$), UBA4334 ($P<0.05$), *Faecousia* ($P<0.05$) and *Acutalibacteraceae* ($P<0.05$) were obviously higher in DFO yaks. In contrast, SFMI01 ($P<0.05$), CAG 273 ($P<0.05$), OLB9 ($P<0.05$), *Akkermansia* ($P<0.05$), *Cryptobacteroides* sp902785575 ($P<0.05$), *Copromorpha* ($P<0.05$), and *Paraprevotella* ($P<0.05$) were observably higher in DFT animals (Fig. 6a,b).

The t-test showed that Firmicutes B 370539 was markedly higher in DFT than in DFO yaks ($P<0.05$) (Fig. 7a). The abundance of *Phocaeicola* A 858004 ($P<0.05$, $P<0.05$), OLB9 ($P<0.05$, $P<0.05$), CAG-41 ($P<0.05$, $P<0.01$), *Cryptobacteroides* ($P<0.01$, $P<0.01$), CAG-273 ($P<0.01$, $P<0.01$) and *Evtepia* ($P<0.05$, $P<0.05$) in DFC yaks was markedly lower than them in DFO and DFT calves, respectively. In contrast, *Faecalimonas* ($P<0.05$, $P<0.05$) was signally higher in DFC yaks. The abundance of *Choladousia* ($P<0.05$) and UMGS1071 ($P<0.05$) was markedly higher in DFC animals than in CFO animals. *Paramuribaculum* ($P<0.05$), CAG-488 ($P<0.01$) and UBA3789 ($P<0.05$) were significantly higher in DFO yak than in DFT animals. UBA5905 ($P<0.05$, $P<0.05$), UBA4334 ($P<0.05$, $P<0.05$) and WQUU01 ($P<0.05$, $P<0.05$) were memorably higher in DFO ruminants than DFC and DFT yaks. UBA737 ($P<0.05$), CAG-269 ($P<0.05$), *Ructibacterium* ($P<0.05$), UBA2658 ($P<0.05$), *Agathobacter* 164119 ($P<0.01$), QAKW01 ($P<0.05$) and *Merdisoma* ($P<0.05$) were signally

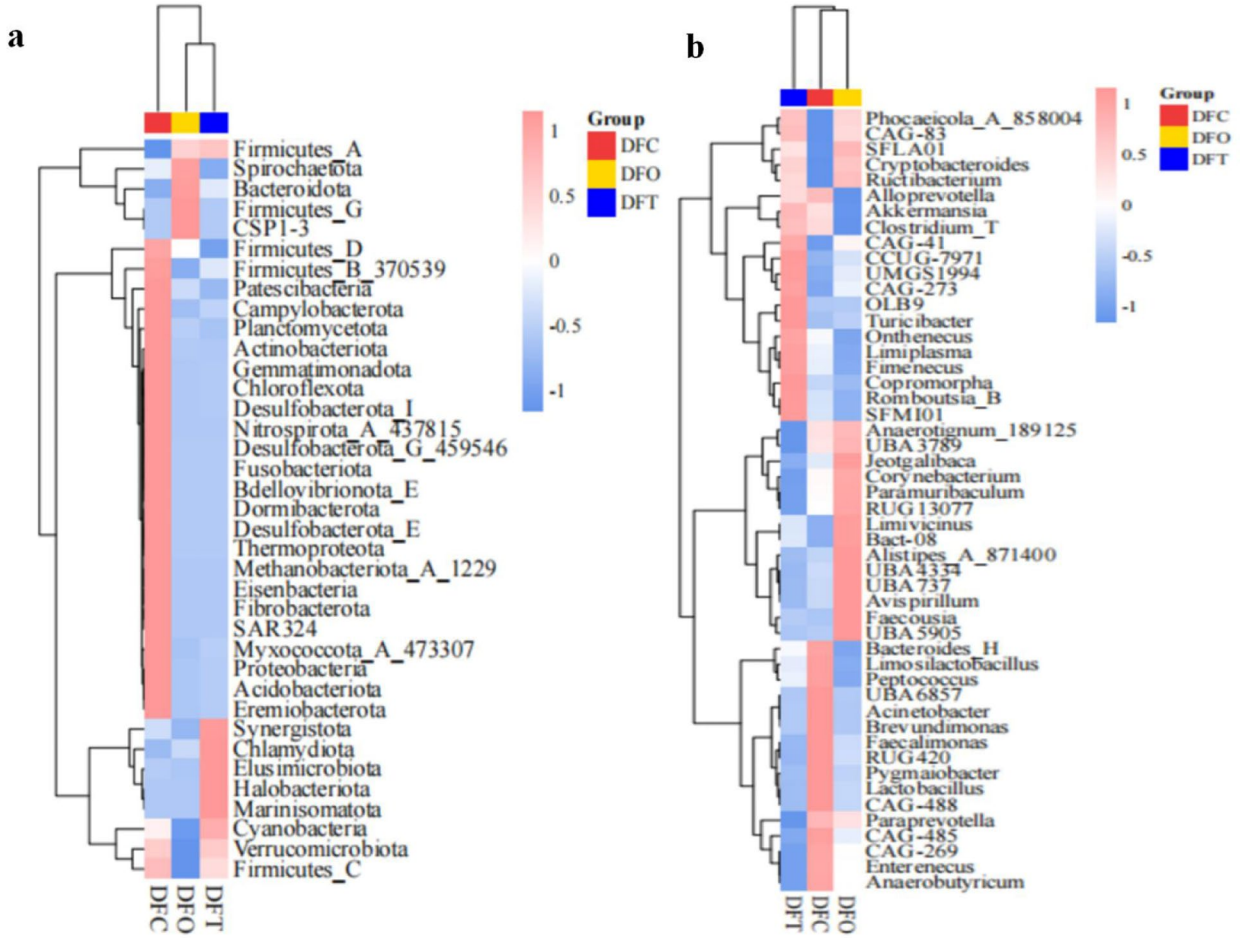

Fig. 5. Heatmap analysis of the top 50 species among yak calves in different groups. (**a**) Phylum, (**b**) Genus.

higher in DFO group than them in DFT group, while *Limiplasma* ($P<0.05$), *Peptococcus* ($P<0.05$), *Bulleidia* ($P<0.01$), UBA9715 ($P<0.05$), UBA11471 ($P<0.01$) and *Saccharofermentans* ($P<0.05$) were obviously lower in DFO animals. CAG-83 ($P<0.01$), SFLA01 ($P<0.01$), CCUG-7971 ($P<0.05$), RF16 ($P<0.05$), and Enterousia ($P<0.05$) were markedly lower in the DFC group than in the DFT group. In contrast, RUG420 ($P<0.05$) and Butyricicoccus A 77030 ($P<0.05$) were observably higher in the DFC group. The abundance of *Anaerobutyricum* ($P<0.05$, $P<0.05$) was obviously lower in yaks in DFT than in animals in DFC and DFT (Fig. 7b).

Discussion

Yaks are economic and religious farm ruminants on the plateau area of China, high-efficient breeding is of utmost importance to local people. Low reproductive performance is a long-time restrictive factor in the development of yak industry, and the long period of yak calves' lactation is an important problem. Here in this study, we explored the effect of starter feeds on early weaning Chawula yak calves to explore novel highly efficient breeding methods in plateau regions.

Starter feeds promoted the growth of Chawula yak clave with observably higher weight ($P<0.05$) and net weight growth rate ($P<0.001$), especially in DFT animals (Fig. 1a), which was in accordance with previous studies[7,19]. Further study indicated that starter feeds enhanced the growth of chest girth in calves in groups DFO ($P<0.05$) and DFT ($P<0.05$) (Fig. 1b). T-AOC, SOD, MDA, and GSH-Px are four core enzyme markers indicating oxidation resistance and oxidative stress state of animals[20,21]. The elevated levels of T-AOC and GSH-Px in starter feeds fed ruminants, especially in calves in DFT groups (Fig. 1c), demonstrated that starter feeds could enhance the antioxidant ability of plateau animals. The improved growth performance observed in DFO and especially DFT calves was closely associated with enhanced antioxidant capacity, as evidenced by higher serum T-AOC and GSH-Px levels, suggesting a reduced oxidative burden that favors nutrient utilization and tissue accretion. Enhanced antioxidant status can support intestinal and ruminal homeostasis, thereby creating a more favorable environment for microbial fermentation and energy harvest. Antioxidant improvement in ruminants is directly linked to positive changes in their gut microbiota, particularly an increase in butyrate-producing microorganisms. Dietary antioxidants modulate the redox balance in the gut, creating a favorable environment that promotes the growth and diversity of beneficial, short-chain fatty acid (SCFA)-producing bacteria such as Lachnospiraceae and Ruminococcaceae families[22]. The increased abundance of these specific

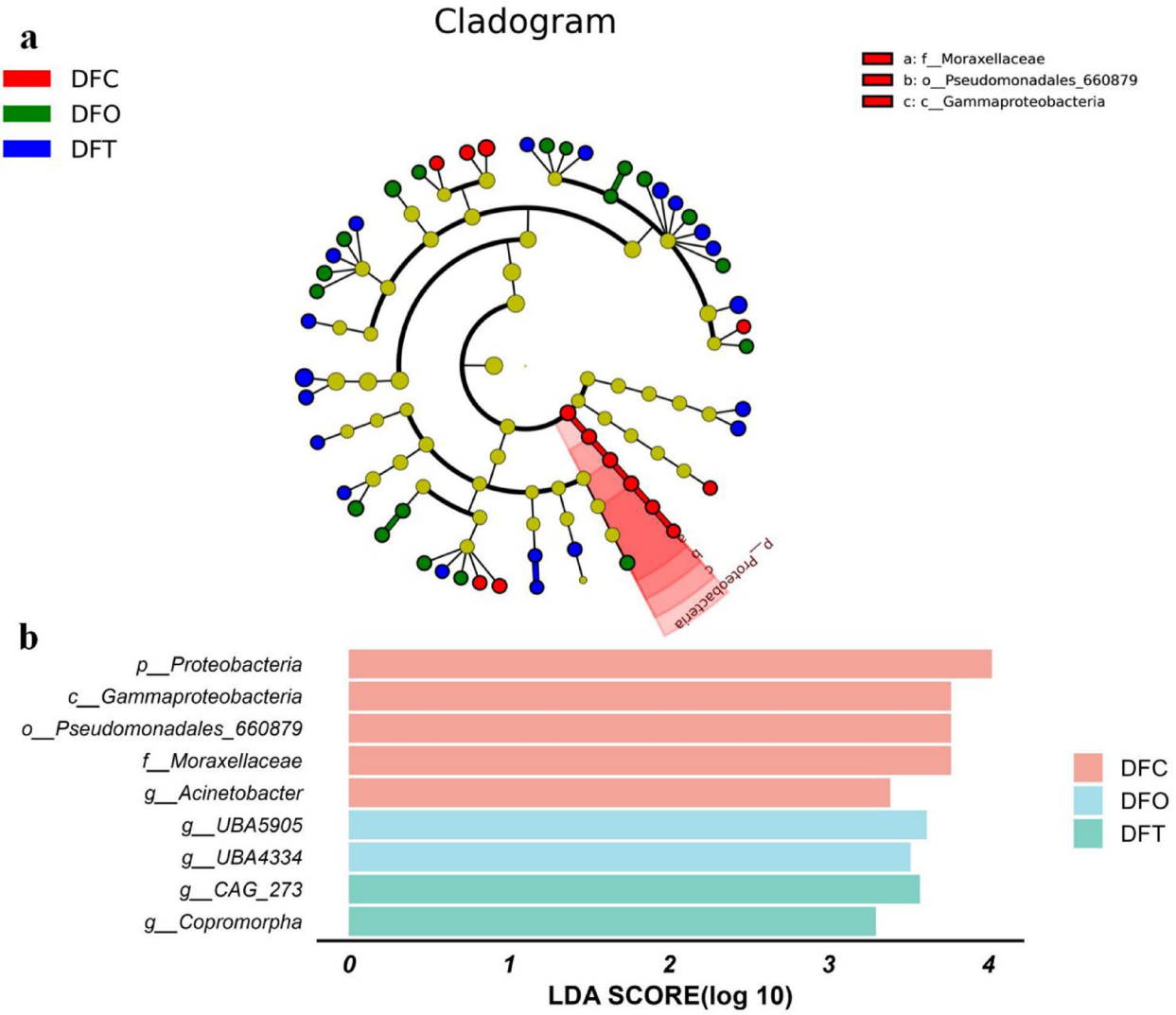

Fig. 6. Biomarkers among yak calves revealed by LEfSe. (**a**) Cladogram diagram, (**b**) Bar chart of LDA effect values for the indicator species.

microorganisms leads to higher concentrations of butyrate, which serves as a primary energy source for colonocytes and plays a critical role in enhancing the ruminant's own antioxidant capacity[23]. This synergistic interaction reduces overall systemic inflammation and oxidative stress, leading to a stronger intestinal barrier, improved nutrient absorption, and better growth performance in ruminants like lambs and calves.

High-throughput sequencing of the microbiota of yak calves in different groups and achieved 391520, 356907, and 353763 filtered sequences in DFC, DFO, and DFT yaks, respectively (Table 1). At the phyla level, the Firmicutes / Bacteroidota value in different groups was 0.40, 0.45, and 0.45 in DTC, DFO, and DFT yaks, which showed slight changes in starter feeds fed yaks, which may indicate the microbiota changes in yaks[24,25]. Then further we explored biomarkers among different Chawula yak calf groups and detected one phylum and thirty-seven different genera in different yak groups (Fig. 7). Among them, higher abundance of *Phocaeicola* A 858004, *Cryptobacteroides* and *Evtepia* was previous reported in yaks with higher weight[26], healthy mice compared with animals with arthritis[27], the higher abundance of those genus in yak calves in DFO and DFT groups may indicate that they are associated with the growth of yak in the plateau. Previous studies found that *Faecalimonas* was associated with skatole formation in pigs[28], inflammatory response in colitis mice[29]. The lower abundance of this genus in supplemented yaks may illustrate that starter feed could inhibit the growth of this negative bacterium. Higher abundance of CAG-83 was found in yaks with higher weights[26], and RF16 in pigs with good growth performance[30], the enrichment of those two genus in DFT yaks may related with the growth promoting effect of starter feed. *Anaerobutyricum* is positively associated with the butyrate generation[31], and butyrate is commonly known to have a favorable function on the homeostasis of the intestine and energy metabolism[32]. However, lower concentration of butyrate was found in lean people compared with obese humans[33]. Further study is needed to explore the relation between Anaerobutyricum and starter feed-treated yaks. Although overall rumen microbial diversity was not altered, distinct shifts in microbial composition and key biomarkers in DFT calves (e.g., enrichment of OLB9, Paraprevotella, and Akkermansia) indicate functional microbial reprogramming rather

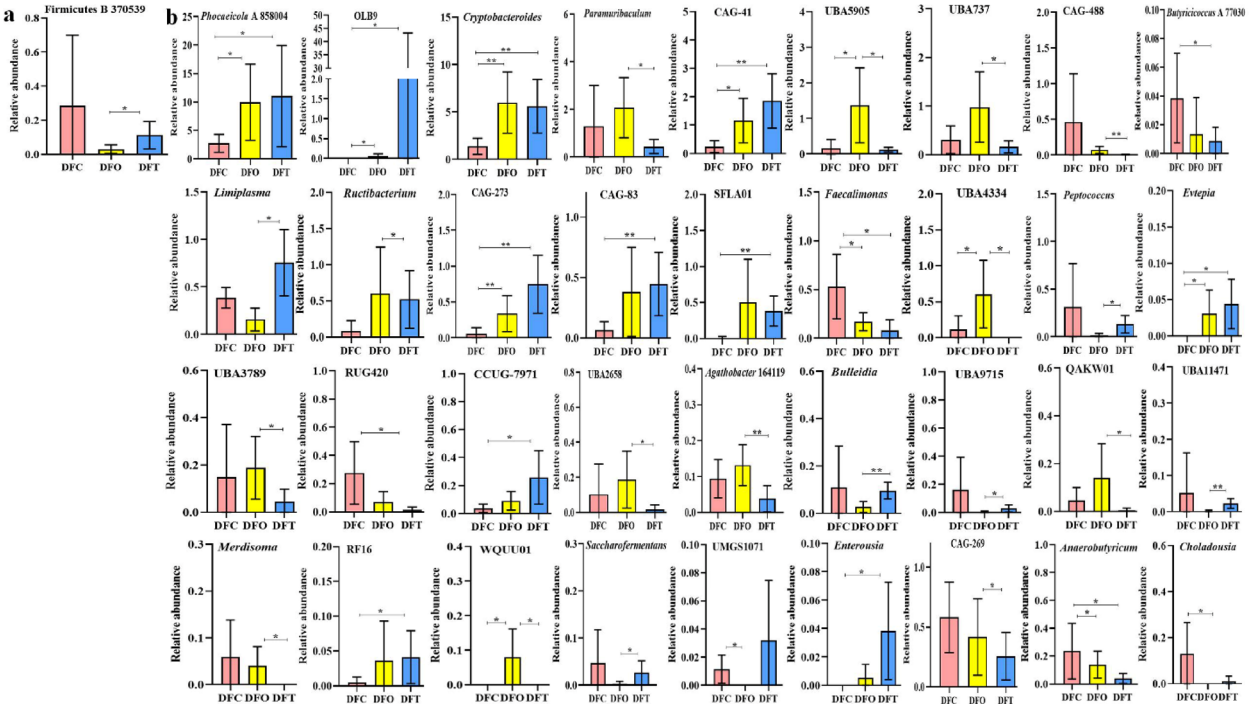

Fig. 7. Biomarkers among yak calves are revealed by T- test. Data are presented as SD, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, and $^{***}P<0.0001$.

than diversity-driven effects. These microbial changes may enhance short-chain fatty acid production, epithelial integrity, and redox balance, collectively contributing to improved growth rates and chest girth development. Together, the results suggest that improvements in antioxidant capacity and rumen microbial composition act synergistically to promote better performance in yak calves.

Conclusion

We concluded that starter feeds could promote the growth of early weaning Chawula yak calves by enhancing antioxidant capacity and regulating the gut microbiota. One phylum (Firmicutes B 370539) and thirty-seven genera (*Phocaeicola* A 858004, *Cryptobacteroides*, *Evtepia*, CAG-273, etc.) were identified as biomarkers in yak calves. Our results may contribute to enhance the breeding efficiency of yaks in the plateau regions.

Materials and methods

Ethics approval

All procedures performed in this research were approved by the Laboratory Animal Welfare and Ethics Committee of Xizang Agricultural and Animal Husbandry University, and Nanjing Agricultural University (NJAU.No20240910164). This study is performed in accordance with relevant guidelines and regulations.

Animal experiment design

Twenty-one male yak calves (3 months) with near weight (22.05 ± 3.05 kg) from a local farm in Nierong County, China, were selected and divided into control group (DFC, n = 7), starter feed group 1 (DFO, n = 7), and starter feed group 2 (DFT, n = 7). Calves in the control group were free-ranged (DFC), the second group were fed with 1.4 kg/head/day alfalfa and 1.4 kg/head/day starter feed 1 (DFO), and third group was fed with 1.4 kg/head/day alfalfa and 1.4 kg/head/day starter feed 2 (DFT) for six weeks. The starter feeds were designed (starter feed 1 containing whey powder (5%), soybean flour (7.5%), fish powder (2.5%), expanded corn (20%), limestone powder (2.5%), calcium hydrogen phosphate (2.5%), salt (0.5%), vitamins (1.5%), additives (8%), and alfalfa (50%), and starter feed 2 containing whey powder (2.5%), soybean flour (3.75%), fish powder (1.25%), expanded corn (10%), limestone powder (1.25%), calcium hydrogen phosphate (1.25%), salt (0.25%), vitamins (0.75%), additives (4%), and alfalfa (75%)) and producted (Item No: 10175852138578) by Yingmeier Ltd., China. Calves' weights and body sizes were measured, and blood samples were taken at three and six weeks of the experiment. At the end of the experiment, fresh fecal samples were collected and stored at − 80 °C.

Antioxidant capacity determination

All of the blood samples of yak calves were centrifuged to get serums for antioxidant capacity examination by employing commercial kits of T-AOC, MDA, SOD, and GSH-px (Nanjing Jiengcheng Bioengineering Research Institute Co., Ltd).

Fecal microbiota sequence

The genomic DNA of calf fecal samples was extracted by employing PureLink™ Microbial DNA Purification Kit (Invitrogen, USA). The quantity and quality of extracted DNAs of yak calves were examined utilizing NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, USA) and 1.5% agarose gel electrophoresis[34,35]. The valid DNA products of calves were employed for 16S rRNA (V3-V4) gene amplification via 338F/806R primer pairs[36], and then those PCR generations were purified and quantified by piloting TIANgel Purification Kit (Tiangen, China) and Quant-iT PicoGreen dsDNA assay (Invitrogen, USA). Finally, amplicons of yaks were sent to pair-end 2 250 bp sequencing utilizing the Illumina MiSeq platform at Bioyi Biotechnology Co., Ltd (Wuhan, China).

Microbiota bioinformatics analysis of yak calves

The generated raw reads were demultiplexed, filtered, denoised, merged, and chimera removed to get quality sequences by employing DADA2[37]. Non-singleton amplicon sequence variants were achieved by aligning yak sequences with MAFIT[38]. The ASVs' taxonomy analysis of yaks was performed by aligning with the Green genes 2 database[39], and a Venn diagram was drawn to visualize co-existing ASVs in different yak groups using the R package[40]. Then alpha (Chao1, Shannon, Simpson, etc.) and beta (Principal coordinate analysis, nonmetric multidimensional Scaling, etc.) diversities of yak calves were calculated via QIIME2[41]. The difference in yaks among different groups was evaluated using PERMANOVA via QIIME2[42]. The biomarkers of yaks in different groups were detected via methods of Linear discriminant analysis, effect size, and T-test[43,44].

Statistical analysis

All of the results of yak calves in starter feed groups were compared with control group by performing student's T-test via SPSS (27.0) to explore the effect of starter feed on animals. Data are presented as means ± SD, and statistical significance is considered when $P < 0.05$.

Data availability

All raw sequence data from weaned yaks were deposited in the NCBI Sequence Read Archive database under accession number: PRJNA1330200.

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Author contributions

DQ, and ZDS: conceptualization and methodology. DQ, PX, HW, KL, SL, and SZL: reagents, materials, and analysis tools. DQ, and ZDS original draft writing and preparation. DQ, DF and ZS: review and editing. ZS: visualization and supervision. All authors reviewed and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

ARRIVE guidelines and consent to participate

All methods are reported by ARRIVE guidelines. This experiment yaks were obtained from a local farm of Nierong County, which is a government owned company, and cooperated with the Xizang Agricultural and Animal Husbandry University. Consent was obtained before the experiment from the manager of this company.

Additional information

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