Introduction

Colon cancer is a kind of digestive system tumors, about 48% of patients with colon cancer are accompanied by anemia, of which 20% of patients even have severe anemia [1]. Colon cancer-related anemia (CCRA) is mainly caused by systemic inflammation, intestinal bleeding, iron deficiency and bone marrow suppression after administration of antineoplastic drugs [2, 3], it combines with aplastic anemia, iron deficiency anemia and hemorrhagic anemia, and iron-deficiency anemia is the most common presenting symptom. Oral or intravenous iron injection, subcutaneous injection of recombinant human erythropoietin (rhEPO) and intravenous
blood transfusion were commonly used in the treatment of CCRA [4], but they also had their own shortcomings. For example, in the treatment of iron deficiency anemia caused by CCRA, oral iron is slow in terms of iron absorption rate, promotes the colon tumor growth and increases metastatic potential [5], some clinical and animal experimental data also shows that rhEPO promotes the growth of tumors [6], and blood transfusion leads to hemolysis, iron overload, thrombosis and so on [7]. To reduce these side effects, more and more therapeutic regimes adopt multi-drug combination, and more and more traditional Chinese medicine is used to treat cancer-related anemia. For instance, Toki-shakuyaku-san resolves the symptoms of mild or moderate anemia associated with uterine myoma without any side effects compared with oral iron [8]. Yixuesheng capsule combined with rhEPO show a better therapeutic effect in treating male neoplastic anemia than that of rhEPO alone [9].

The severity of CCRA is closely related to immunity, nutritional composition and iron metabolism [10], so the therapeutic regime of CCRA should focus on these three points. Danggui Buxue Decoction (DBD) was a classical prescription for tonifying qi and enriching blood. Our previous studies have shown that DBD have significant therapeutic effects on hemorrhagic anemia and aplastic anemia, and improve iron binding and immunity. DBD and iron supplementation had a strong ameliorating effect on iron-deficiency anemia rats by a 2-hour interval of DBD administration prior to ferrous sulfate treatment [11]. In this study, DBD was employed to play a synergistic role for the treatment of CCRA in combination with Fe and EPO, specific performances: rhEPO promotes hematopoiesis, Ferrum Hausmann provides iron, and DBD improves hematopoiesis, iron absorption and immunity. Recently, advancements in genome sequencing technologies, bioinformatics and culturomics have led to the groundbreaking characterization of the gut microbiota, a “forgotten organ”, and its role in host health and disease [12]. Iron was critical for the replication and survival of almost all bacteria, with a few exceptions, which acquired alternative metabolic solutions from evolution [13]. Based on a great deal of evidence, iron deficiency anemia may be featured by unfavorable changes of the gut microbiota no matter in animals or humans [14-19], however, the gut microbial changes of CCRA have not been reported. The changes of gut microbiota were detected with Illumina MiSeq sequencing in this study, and our work may provide a basis for further study about the CCRA model building, clinical application of blood tonics and disturbance of gut microbiota in CCRA.

Materials and Methods

**Chemicals and reagents**

The radix of Angelica sinensis (Oliv.) Diels (Apiaceae) (AS) was collected at Min County, Gansu Province, China, in November, 2017. The radix of Astragalus membranaceus (Fisch.) Bge. var. mongholicus (Bgr.) Hisiao (Fabaceae) (AR) was collected at Inner Mongolia, China, in January, 2018. They were identified by Dr. YAN Hui (Department of Pharmacognosy, Nanjing University of Chinese Medicine, Nanjing, China) and were up to the standard of Chinese Pharmacopoeia. The voucher specimens (No. NJUCM-20171124 and NJUCM-20180116) were deposited in the Herbarium of Nanjing University of Chinese Medicine. CT-26 cells were obtained from the Cell Bank of the Chinese Academy of Sciences. EDTA-2Na was offered by Shanghai Danning Chemical Co., Ltd. (Shanghai, China). Ferrum Hausmann (Fe) was purchased from Vifor Pharma (Madison, Germany). rhEPO was purchased from 3S BIO INC (Shenyang, China). Other reagents and chemicals were all analytical grade.

**Animals and drug administration**

**Modeling:** The CT-26 cells were routinely cultured in RPMI1640 medium containing 10% fetal bovine serum (FBS; BI, Israel), 100 U·mL⁻¹ Penicillin and 100 μg·mL⁻¹ streptomycin at 37 °C in a 5% CO₂ incubator. Tumors were generated by the subcutaneous implantation of CT-26 cells (2×10⁶ cells) between right forelimb and right hind limb of mice [20]. A week after implantation, when tumors reached a volume of about 150 mm³ (calculation formula of tumor volume: tumor volume = 0.5 × length × width²), tumor-bearing mice were i.p. with a dosage of 5 mg·kg⁻¹ of oxaliplatin or received no treatment every other day (Day 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27). The CCRA model was built with CT-26 cells inoculation and oxaliplatin treatment and the period was 28 days.

**Medicine Preparation:** A total 600 g mixed pieces of AR-AS (5 : 1, W/W) were decocked with boiling water for 2 h (1 : 8, W/F), and then the gruffs decocted twice with water (1 : 6, W/V), 1.5 h for each time, filtered through gauze. The three filtrates were merged and evaporated by rotary evaporation under vacuum at 60 °C. The rhEPO injection was diluted with sterile saline, oxaliplatin sterile powder was dissolved with sterile glucose injection, and Fe was diluted with ultrapure water.

**Feeding and grouping:** BALB/c male mice (18−22 g, 20180006003844) were purchased from Sino-British SIPPR/BK Lab. Animal Ltd. (Shanghai, China) (SCXK 2018-0006), and kept in an environmentally controlled breeding room (temperature: 20 ± 2 °C, humidity: 60% ± 5%). Animal welfare and experimental procedures were strictly in accordance with the Guide for the Care and Use of Laboratory Animals (US National Research Council, 1996) and the related ethics of Nanjing University of Chinese Medicine (Ethical Number: 202001A003). After 10 days for acclimatization, 81 BALB/c male mice were randomly and equally divided into 9 groups, following as Control (C), Colon cancer (A), CCRA (P), rhEPO (E), rhEPO + DBD high dosage (ED), rhEPO + DBD high dosage + Fe (EDF), DBD high dosage (D-H) and DBD low dosage (D-L) groups. Mice in rhEPO-contained groups were s.c. rhEPO at a dosage of 1500 U·kg⁻¹ every other day (Day 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28); mice in D-H-contained and D-L-contained groups were i.g. DBD at a dosage of 14.04 and 9.36 g·kg⁻¹ everyday (Day 1−28), respectively; mice in Fe-con-
tained groups were i.e. Fe at a dosage of 50 mg kg\(^{-1}\) every-day (Day 1–28), but needed 2-hour interval of DBD administration prior to Fe. The modeling and administration were proceeded at the same time.

**Pharmacodynamic study**

Mice were weighed twice a week and the number of deaths was recorded at any time. The tumor volume was also measured with caliper (tumor volume = 0.5 × length × width) every other day. According to the weight of the tumors taken on the last day, we calculated the weight of tumors per unit volume, and the body weight curve of the mice after removing tumors was drawn. After 30 min of the last administration, 0.5 mL blood samples were collected into the centrifuge tubes which contained 50 μL 15 mg mL\(^{-1}\) EDTA-2Na. 0.1 mL whole blood samples were applied to determine the peripheral blood indexes, including hemoglobin (HGB/g·L\(^{-1}\)), red blood cell count (RBC/10\(^{12}\)·L\(^{-1}\)), white blood cell count (WBC/10\(^{9}\)·L\(^{-1}\)), haematocrit (HCT/%) and reticulocyte count (RTC/10\(^{12}\)·L\(^{-1}\)), remaining whole blood samples were centrifuged at 3 000 r·min\(^{-1}\) for 10 min, and the plasma samples were collected into new tubes. Thymus, spleen, liver, kidney and tumor samples were taken and weighed. According to organ index formula (organ index (g/g) = organ weight (g) / body weight (g)), thymus, spleen, liver and kidney indexes were calculated. The colon contents were also collected in frozen pipes. Femur were processed by 4% formamint-fixed and pathological changes were observed by H&E staining techniques. All of samples were kept at −80 °C until analysis.

**Microbial study**

DNA extraction and PCR amplification [21]

Microbial DNA was extracted from colon content samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer’s protocols. The final DNA concentration and purification were determined by NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The V3-V4 hypervariable regions of the bacteria 16S rRNA gene were amplified with primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) by thermocycler PCR system (Gene Amp 9700, ABI, USA). The PCR reactions were conducted using the following program: 3 min of denaturation at 95 °C, 28 cycles of 30 s at 95 °C, 30 s for annealing at 55 °C, and 45 s for elongation at 72 °C, and a final extension at 72 °C for 10 min. PCR reactions were performed with TransStart Fastpfu DNA Polymerase in triplicate 20 μL mixture. PCR products were extracted from a 2% agarose gel and further purified by the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified by QuantiFluor-STM-P (Promega, USA).

**Illumina MiSeq sequencing and data processing** [22]

Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Raw fastq files were quality-filtered by Trimmmatic and merged by FLASH with the following criteria: (i) The reads were truncated at any site receiving an average quality score < 20 over a 50 bp sliding window. (ii) Sequences whose overlap being longer than 10 bp were merged according to their overlap with mismatch no more than 2 bp. (iii) Sequences of each sample were separated according to barcodes (exactly matching) and Primers (allowing 2 nucleotide mismatching), and reads containing ambiguous bases were removed. Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (Version 7.1, http://drive5.com/uparse/) with a novel “greedy” algorithm that performs chimera filtering and OTU clustering simultaneously. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier algorithm (http://rdp.cme.msu.edu) against the Silva (SSU123) 16S rRNA database using confidence threshold of 70%. Distance matrix was visualized with principal coordinate analysis (PCoA) [22]. Finally, 851 OUT were obtained, followed as: Domain 1, Kingdom 1, Phylum 11, Class 19, Order 37, Family 66, Genus 162, Species 270. The optimizing sequences, optimizing bases and averages length of samples were 1959866, 81663805 and 417.7010. The length of trimmed sequences was between 400-500 bp, and the results indicated that the 16S rRNA sequences identified in the groups represent the majority of bacteria present in the study samples. (Supplementary Fig. 1).

**Statistics**

Statistical analysis was performed using Graphpad Prism 7.0 software, all data were expressed as mean ± standard deviation. The statistical results were conducted with Newman-Keuls Multiple Comparison Test in ANOVA, and a P-value of <0.05 was considered statistically significant.

**Results**

**Pharmacodynamics results**

Mortality and weight

During the experimental period, there were only two mice of D-L group dead in day 22 and day 28. The body weight of mice in C group showed a relatively gentle growth trend throughout the experimental cycle (Fig. 1A). Except for C group, the body weight of mice in other groups were all decreased in the first week, especially the mice in P group. The body weight of mice in each group increased slowly from second to third week. The body weight of mice in each group showed a steeper increase trend in fourth week. Fig. 1B showed the body weight of mice in each group after minus tumor weight. It could be seen that in first week, the body weight in each group decreased rapidly except for C group, and there was a slowly declined trend between the second week and the third week. In day 22, the weight dropped to the lowest point, and in the last week, the weight of mice in C and ED groups increased firstly after declining and the other groups increased continuously.

**Tumor volume**

The tumor volume of mice in each group shown in Fig. 2. In Fig. 2A, the tumor volume of mice in oxaliplatin treat-
ment groups were significantly smaller than A group, which indicated that oxaliplatin had significant antitumor activity. In Fig. 2B, the tumor growth rate in DF group was the fastest from the first to 9th day, however, that was slowest in D-L group. On the 17th to 29th day, the tumor volume of D-H group and D-L group increased slowly, however, it was much smaller than that of P group; the tumor volume of EDF group was similar to that of P group; the tumor volume of E group, DF group and EPD group was much larger than that of P group. The results indicated that EDF, D-H and D-L might have anti-tumor effect.

Peripheral blood indexes and organ indexes analysis

The peripheral blood indexes were showed in Fig. 3. Compared with C group, all of the indexes were decreased significantly in P group, which indicated that the model of CCRA was established successfully. Compared with P group, all of the indexes in ED group and EDF group were increased significantly, and E group showed significantly increasing in RBC, HGB, HCT and RTC; however, the significant increase in DF group, D-H group and D-L group was only reflected in RTC. The significantly decrease WBC in P group indicated that oxaliplatin might reduce the defense function of the body, and EDF group showed the strongest defense function. The low RTC indicated that hematopoietic function was suppressed in P group, all of the treatment groups showed hematogenic function, and ED group was the strongest.

Fig. 1 The body weight of mice in each group (A); The body weight of mice in each group after minus tumor weight (B)

Fig. 2 The tumor volume of mice in each group (A); The tumor volume of mice in oxaliplatin treatment groups (B)

Fig. 3 Peripheral blood indexes of different groups (X ± S). C vs P, *P < 0.05, **P < 0.01; P vs treatment groups, ^P < 0.05, ^^P < 0.01
The organ indexes were showed in Fig. 4. Compared with C group, all of the indexes were changed significantly in P group, which indicated that after modeling, spleen, liver and kidney were enlarged to varying degrees, on the contrary, the thymus atrophied. Compared with P group, all of the treatment groups could significantly alleviate splenomegaly, but had no effect on liver. Thymus index of E group, ED group, D-H group and D-L group increased significantly, and except for D-H group and D-L group, the other four groups could significantly decrease the kidney index. The results indicated that oxaliplatin showed significantly thymus, spleen, liver and kidney injury. All of the treatment groups showed no significant protective effect on liver, but had a certain protective effect on thymus, spleen and kidney. ED group and E group showed the best effect on organ protection.

**Microbial results**

**Gut microbial richness, evenness and diversity in different groups**

From the pharmacodynamics results, we could see that compared with other treatment groups, only EDF group and ED group showed significantly blood enrichment effect. Thus, gut microbiota modulation only studied the ED and EDF group. The maintenance of sufficient bacterial richness and diversity is important for providing the gut microbiota with functional redundancy, adaptability and systematic robustness in the face of environmental challenges. The alpha diversity indexes of OTU level were calculated with mothur (version v.1.30.1, http://www.mothur.org/wiki/Schloss_SOP#Alpha_diversity). Sobs, Shannon and Heip indexes reflected the richness, diversity and evenness of biological communities, respectively [23]. In Fig. 6A–6C, the three indexes were all showed no significant difference between groups, however, Sobs index of EDF group was little higher than the other three groups, which indicated EDF could increase the richness. ED group showed lower Sobs, Shannon and Heip indexes, indicating that ED could decrease the richness, diversity and evenness of gut microbiota. The Sobs, Shannon and Heip indexes between C group and P group showed little difference, suggesting that CCRA might have little change on richness, diversity and evenness. The rank-abundance curves displayed the microbial community’s richness at horizontal direction and evenness at vertical direction, from Fig. 6D, EDF group showed a higher richness and evenness.

Beta diversity is the amount of variation in species composition among sampling units [24]. Beta diversity of the microbiota was assessed by principal coordinate analysis of the weighted UniFrac Principal Component Analysis (PCoA) [25] and hierarchical clustering tree. Hierarchical clustering tree on OTU level was showed in Fig. 7A, C group and P group clustered respectively and EDF group and ED group clustered together. In Fig. 7B, C group and P group was separated obviously, indicating that the gut microbial beta diversity was

![Fig. 4](image-url)  **Organ indexes of different groups (X ± S). C vs P, *P < 0.05, **P < 0.01; P vs treatment groups, #P < 0.05, ##P < 0.01**
wrecked in CCRA mice. EDF group and ED group were closer to the C group in distance, and EDF group showed shorter distances, which indicated that EDF and ED could improve the flora imbalance and EDF showed better effect. Analysis of similarity (ANOSIM) with 999 permutations was used to give more evidence to the separation of 4 groups with distance matrices of weighted UniFrac. ANOSIM revealed significantly separating between C group and P group ($R = 0.8076; P = 0.001$), C group and ED group ($R = 0.7852; P = 0.001$), and C group and EDF group ($R = 0.5471; P = 0.003$). The closer the $R$ value was to 1, the greater the difference between groups was, and the results indicated that EDF might
have a better treat effect. Differentially abundant bacterial groups in mice following CCRA

In order to further identify the species that lead to significant inter-group variations, the bacteria composition analysis was firstly carried on. In phylum level (Fig. 8A), Bacteroidetes, Firmicutes, Verrucomicrobia, Proteobacteria, Epsilonbacteraeota and Patescibacteria were the six major phyla present in the colonic microbiota, among them, Bacteroidetes, Firmicutes and Verrucomicrobia were the dominant genus accounting for more than 80% of total gut bacteria. The Firmicutes/Bacteroidetes ratio in P group was higher compared with C group with significant difference, and EDF group and ED group was significantly lower than P group, and EDF group showed a lower trend (Fig. 8B). As shown in Fig. 8C, the Kruskal-Wallis H test was applied to compare the difference among the 4 groups, the top 7 phyla were all showed significant change between groups. Compared with P group, the relative abundant of Bacteroidetes on EDF group and ED group was increased, and the other phyla were decreased, however, the relative abundant of Epsilonbacteraeota on ED group was obvious increase, and Patescibacteria was decrease. The relative abundance of bacteria at genera level was also analyzed, and finally, 23 mainly genera were detected after merging those relative abundance less than 0.01 in the colonic fecal pellet (Fig. 8D). Among them, norank_f_Muribaculaceae, Lachnospiraceae_NK4A136_group, Bacteroides, Lactobacillus, Helicobacter, unclassified_f_Lachnospiraceae and Odoribacter were the dominant genera accounting for more than 60% of total gut bacteria. The between groups difference in genus level were also calculated and the top 20 were showed in Fig. 8E. Most of the genera belonged to Bacteroidetes, and norank_f_Muribaculaceae was the most abundant genus. EDF and ED could increase the relative abundant of Bacteroides, norank_f_Muribaculaceae, Alloprevotella, Prevotellaceae_UGC-001 and Parabacteroides, and decrease the relative abundant of Odoribacter and Alistipes in CCRA mice. Lachnospiraceae_NK4A136_group, unclassified_f_Ruminococcaceae, Lactobacillus, unclassified_f_Lachnospiraceae, Ruminococcaceae_UGC-014 and Lactococcus were all part of Firmicutes, except for Lactococcus, the other genera were decreased in EDF group and ED group. In addition, Candidatus_Saccharimonas of Patescibacteria, Akkermansia of Verrucomicrobia, Helicobacter of Epsilonbacteraeota and Escherichia-Shigella of Proteobacteria were also showed with varying degrees of change among 4 groups.

16S rRNA sequencing analysis

16S function prediction was conducted using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) software and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, according to the tutorial on the official website (http://picrust.github.io/) [26]. Finally, 213 KEGG pathways were obtained, by comparing relative abundance between groups, 49 metabolic pathways which showed significant difference between C and P groups were retained, the results were showed in Supplementary Table 1, mainly including citrate cycle (TCA cycle), pentose phosphate pathway, oxidative phosphorylation, alanine, aspartate and glutamate metabolism, histidine metabolism, one carbon pool by folate, carbon fixation pathways in prokaryotes, terpenoid backbone biosynthesis, ABC transporters, two-component system, ribosome, protein export, ascorbate and aldarate metabolism, and so on. In Fig. 9, the representative KEGG pathways changed significantly were displayed. In P group, B vitamins metabolism was decreased compared with C group. Compared with P group, thiamine metabolism, vitamin B6 metabolism and nicotinate and nicotinamide metabolism were significantly increased in EDF group, and riboflavin metabolism, pantothenate and CoA biosynthesis, biotin metabolism and folate biosynthesis were significantly increased in ED group. In energy metabolism, oxidative phosphorylation was significantly increased only in ED group, and TCA cycle were both significantly increased in the two treatment groups. Furthermore, compared with ED group, glutamate metabolism, one carbon pool by folate and cysteine and methionine metabolism in EDF group was significantly increased, while lipopolysaccharide biosynthesis was significantly decreased.

Discussion

Cancer patients often showed low immunity, anemia, etc. which seriously affected the therapeutic effect of cancer and patients’ daily life. In this study, DBD, rhEPO combined with
Fig. 8   The community barplot analysis of 4 groups on phylum (A) and genus (D) level; The Wilcoxon rank-sum test bar plot of 4 groups on phylum (C) and genus (E) level; The Firmicutes/Bacteroidetes ratio of 4 groups (B). C vs P, *P < 0.05, **P < 0.01
Fe were employed to explore the blood enrichment and anti-tumor effects in CCRA mice. From the pharmacodynamics results, only ED and EDF showed significant blood enrichment effect, and ED showed the better effect. In P group, *Lachnospiraceae_NK4A136* group, unclassified_f_Lachnospiraceae, *norank_f_Lachnospiraceae*, *Lactobacillus* and *Odoribacter* showed higher abundance, and EDF and ED both decreased the trend at a similar level. *Lactobacillus* showed higher abundance in iron deficiency pig colon 

Odoribacter is known to be opportunistic pathogens in circumstances of intestinal barrier destruction and the abundance of Odoribacter may increase in the murine colon after oxaliplatin treatment. The results indicated that ED and EDF might enhance the immunity of CCRA mice by regulating *Lactobacillus* and *Odoribacter*.

However, ED group showed larger tumor volume than P group and EDF group. Compared with EDF group, ED group showed higher abundance of *Akkermansia*, *Helicobacter*, *Escherichia-Shigella*, *Alloprevotella*, *Parabacteroides* and *Lactococcus*, while lower abundance of *Prevotellaceae_UCG-001* and *Ruminococcaceae_UCG-014*. *Escherichia-Shigella* and *Parabacteroides* were both belonged to Proteobacteria which seemed as the imbalance-induced gut microbiota. *Escherichia-Shigella* was a pro-inflammatory gut microbiota taxon, and the low abundance in EDF group indicated that EDF might have a better anti-inflammatory effect. *Helicobacter* and *Lactococcus* were harmful bacteria which had been confirmed to be positively associated with colorectal cancer and *Alloprevotella* also seemed as a cancer-related bacterium. The decreasing abundance of *Akkermansia* in EDF group might increase the thickness of mucus layer and alleviate the damage of intestinal mucosal barrier. The increased glutamate metabolism and high abundance *Ruminococcaceae* in EDF group might induce the production of butyrate which seemed as the be the protective compound in colon cancer. The decreased lipopolysaccharide biosynthesis and increased cysteine and methionine metabolism in EDF group might protect the tissue from the damage of oxaliplatin. The smaller tumor volume in EDF group might be related to the lower abundances of *Akkermansia*, *Helicobacter*, *Escherichia-Shigella*, *Alloprevotella*, *Parabacteroides* and *Lactococcus* and higher abundance of *Ruminococcaceae_UCG-014*.

**Conclusion**

In this study, we successfully established colon cancer-related anemia mice model, which might provide a new method to the study of CCRA. Our findings proposed the best treatment combination of DBD, rhEPO and Fe in CCRA and provided theoretical basis and literature reference for CCRA-induced intestinal flora disorder and the regulatory mechanism of EDF.

**Abbreviations**

CCRA: colon cancer-related anaemia; DBD: Dongguibuxue decoction; ED: rhEPO + DBD high dosage + Fe; ED: rhEPO + DBD high dosage; CRA: cancer-related anaemia; rhEPO: recombinant human erythropoietin; TCM: traditional Chinese medicine; AS: Angelica sinensis (Oliv.) Diels (Apiaceae); AR: Astragalus membranaceus (Fisch.) Bge. var. mongholicus (Bgr.) Hsiao (Fabaceae); C: control; A: colon cancer; P: CCRA; E: rhEPO; DF: DBD high dosage + Fe; D-H: DBD high dosage; D-L: DBD low dosage; HGB: hemoglobin; RBC: red blood cell count; WBC: white blood cell count; HCT: haematocrit; RTC: reticulocyte count; OTUs: operational taxonomic units; PCoA: principal coordinate analysis; PICRUSt: Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; KEGG: Kyoto Encyclopedia of Genes and Genomes; TCA cycle: citrate cycle.

**References**


