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**Effects of concentrate feeding sequence on growth performance, feed digestibility and gut microorganisms of weaned donkeys**

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**Abstract:**

**Background/Objective:** The Dezhou donkey is a superior indigenous breed in China, with excellent productivity and high forage utilization rate. There are few reports on nutritional recommendations and optimal feeding practices for donkeys. Therefore, it is significant to explore the appropriate feeding methods to improve the production efficiency of donkeys.

**Methods:** We measured nutrient digestion by the acid-insoluble ash method and analyzed the fecal microbiota of the weaned donkeys by high-throughput sequencing of 16s rRNA genes in the V3-V4 region. Non-targeted metabolomics was studied based on LC-MS technology to analyze fecal metabolites of weaned donkeys.

**Results:** The results indicate that group C3 exhibited superior growth performance, with significantly higher digestibility of crude protein (CP) and crude extract (EE) compared to group C1 (*P* < 0.05). Acetic acid, isobutyric acid, valeric acid, isovaleric acid, and caproic acid were notably different among all groups (*P* < 0.05). In addition, *Firmicutes* and *Bacteroidetes* were dominant in the fecal microbes of each group, and *Firmicutes* was significantly higher in group C3 (*P* < 0.05). At the genus level, the different genera were *Treponema*, *RikenellaceaeRC9-gut-group, Unidentified-F082*, and *Bacteroidales-RF16-group* (*P* < 0.05). The prediction of fecal microbiota function by PICRUSt indicated that different feeding sequences had minimal impact on the function of the fecal microbiota, particularly on the high-abundance pathway. Concentrate feeding sequence had a significant effect on fecal metabolites. A total of 243 differential metabolites were identified, including 181 in positive ion mode, of which 82 were significantly up-regulated and 99 were significantly down-regulated. In the negative ion mode, 29 of 62 were significantly up-regulated and 33 were significantly down-regulated. These different metabolites are significantly enriched in bile secretion pathway, glycerol phospholipid metabolic pathway, choline metabolic pathway, aminoacyl-trNA biosynthesis, protein digestion and absorption, amino acid biosynthesis, histidine metabolism and other pathways.

**Conclusion:** The growth performance of weaned donkey foals was significantly influenced by the sequence of concentrate feeding, and the optimal growth performance was observed when they were fed a combination of concentrate and coarse mixed diet. The order of concentrate feeding affected the growth performance by regulating the composition of intestinal microorganisms. The increase of microorganisms and the enrichment of metabolite pathway were positively correlated with the average daily gain of donkeys. In conclusion, the total mixed diet enhances the abundance of beneficial bacteria, consequently altering the metabolic pathway associated with growth and ultimately contributing to improvements in growth performance and feed efficiency in donkeys. These beneficial bacteria and metabolic pathways associated with feeding patterns are potential targets for regulating growth performance.

**Keywords:** Weaned donkeys; Concentrate feeding sequence; Growth performance; Gut microbes; VFA.