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**Effects of Concentrate feeding Sequence on VFA production, and Cecal microbiota of Dezhou donkeys by Metagenomic technology**

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**Abstract:**

The rapid development of large-scale donkey breeding does not match the supporting breeding management technology. At present, most of them draw on the experience of large-scale cattle and sheep breeding, but there are significant differences between donkeys and cattle and sheep in terms of behavioral habits and digestive physiology. Therefore, it is necessary to explore suitable diet formulations and feeding methods as soon as possible. Fifteen Dezhou donkeys with similar age and weight profiles were randomly divided into three groups with the concentrate feeding sequence: fiber-to-concentrate (FC), concentrate-to-fiber (CF), and total mixed ration (TMR). The experiment spanned a duration of 82 days. The analyses conducted were primarily aimed at determining the effects of feeding on gut microbes, primarily using metagenomic sequencing techniques.The experimental findings revealed that the levels of valeric acid were notably higher in the CF and TMR groups compared to the FC group (*P*<0.05). At the phylum level, the predominant microbiota consisted of Firmicutes and Bacteroidetes, with the CF group displaying a higher relative abundance of Firmicutes compared to both the FC and TMR groups. At the genus level, *Prevotella*, *Bacteroides*, and *Fibrobacter* were the dominant bacterial genera identified in cecum. The functional gene annotation analysis indicated a significantly lower abundance of *lacZ* (K01190), *Por/nifJ* (K03737), and *ppdK* (K01006) genes in CF group relative to the FC and TMR groups (*P*<0.05). Moreover, the CF group exhibited a higher concentration of antibiotic resistance genes (*tetO* and *tet44*) in the gut microbiota compared to the TMR and FC groups (*P*<0.05). In conclusion, different precision feed sequences significantly impact the levels of volatile fatty acids in Dezhou fattening donkeys, modify the composition and functional genes of the cecal microbiota, and elucidate the microbial mechanisms influenced by the feeding sequence on the growth and metabolism. These insights are anticipated to provide a foundation for the rational design of precision feed sequences in practical agricultural settings.

**Keywords:** Dezhou Donkeys; Cecal microbiota; Metagenomic Technology; Concentrate Feeding Sequence; VFA