

# Gut Microbiota Response to Alfalfa Leaf Meal Improves Meat Quality by Modulating Lipids Metabolism in Finishing Pigs

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**Keywords:** alfalfa leaf meal; finishing pigs; gut microbiota; meat quality; lipids metabolism

With the rising price and shortage of soybean meal, protein resources are emerging as a serious issue. Therefore, exploitation and utilization of new protein resources becomes an urgent. To further investigate the feasibility and effectiveness in using alfalfa leaf meal (ALM), 64 finishing pigs with similar body weight were randomly assigned to 4 groups. Each group was received diet replacing 0% (Ctrl), 25% (25% ALM), 50% (50% ALM), 75% (75% ALM) soybean protein with ALM, respectively. The growth performance, meat quality, gut microbiota, and metabolic profiles of *longissimus dorsi* muscle were detected. The results showed that 25% ALM significantly improved the growth performance and meat quality of pigs. In comparison with Ctrl, daily gain was not altered by 25% ALM, whereas 25% ALM markedly decreased F/G (feed/gain) and significantly enhanced carcass weight and dressing percentage, respectively. Interestingly, fat content was significantly increased in 25% ALM and 50% ALM when compared to Ctrl. In addition, palmitoleic acid and  $\alpha$ -linolenic acid were significantly enhanced while stearic acid was significantly reduced in 25% ALM, respectively, in *longissimus dorsi* muscle of the finishing pigs. Metabolome analysis showed that 25% ALM altered the metabolic profiles of muscle, especially lipid and amino acids related metabolites. Furthermore, gut microbiota composition also altered in 25% ALM. At the phylum level, 25% ALM significantly *Campilobacterota* increased in ileum, while *Firmicutes* decreased in colon. At the genus level, 25% ALM significantly increased the relative abundances of *Romboutsia*, *Helicobacter*, and *Corynebacterium* ileum; 25% ALM significantly increased the relative abundances of *Sphaerochaeta* in colon. Our data also demonstrated that the fat deposition related genes CD36 (lipid transport), PPAR $\alpha/\delta$ , SREBP1, and ACC $\alpha$  (lipogenesis) in *longissimus dorsi* muscle from 25% ALM were significantly increased. Correlation analysis showed the enriched microbiota in 25% ALM were positively correlated with muscle fat content and serum TG content. These results reveal gut microbiota response to 25% ALM improves meat quality by regulating lipid metabolism in finishing pigs and also provide a theoretical basis and guidance for the use of novel protein sources.

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