Effect of Alfalfa Meal on Meat Quality, Muscle Amino Acid and Fatty Acid Content in Finishing Pigs (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary alfalfa meal supplementation on growth performance, meat quality, and muscle amino acid and fatty acid contents in finishing pigs. A total of 130 healthy "Landrace × Large White" or "Large White × Landrace" crossbred finishing pigs with an average body weight of (60.28$±$0.73) kg were randomly allocated to 5 groups with 26 replicates per group and 1 pig per replicate. The control group was fed a basal diet, while the treatment groups were fed the basal diet supplemented with 5%, 10%, 20%, and 30% alfalfa meal, respectively. The experiment consisted of a 7-day adaptation period followed by a 72-day experimental period. The results showed that: 1) The 5% alfalfa meal group exhibited the highest average daily gain (ADG) and the lowest cost per kg gain. 2) Dietary alfalfa meal supplementation had no significant effect on muscle pH at 45 min, cooking yield, marbling, or meat color (P>0.05). Compared with the control group, muscle pH at 24 h was highly significantly decreased in all alfalfa meal groups (P<0.01). The 30% alfalfa meal group showed significantly lower drip loss than the control and 5% alfalfa meal groups (P<0.05). 3) Compared with the control group, the 20% alfalfa meal group exhibited significantly increased contents of delicious amino acids (DAA) (aspartic acid, glutamic acid, glycine, alanine), essential amino acids (EAA) (methionine, valine, isoleucine, phenylalanine, leucine, threonine, lysine), histidine, arginine, tyrosine, serine, proline, and total amino acids (TAA) (P<0.05). 4) As the dietary alfalfa meal level increased, muscle saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) contents gradually decreased, while muscle unsaturated fatty acid (UFA), polyunsaturated fatty acid (PUFA), n-6 PUFA, and n-3 PUFA contents gradually increased. Compared with the control group, the 20% and 30% alfalfa meal groups showed significantly lower muscle MUFA and oleic acid contents (P<0.05) and significantly higher muscle UFA content (P<0.05). The 20% and 30% alfalfa meal groups had

significantly higher muscle PUFA, linoleic acid, α-linolenic acid, n-6 PUFA, and n-3 PUFA contents than the control, 5%, and 10% alfalfa meal groups ($P<0.05$). The muscle n-6 PUFA/n-3 PUFA ratio was highly significantly lower than that in the control, 5%, and 10% alfalfa meal groups ($P<0.01$). In conclusion, dietary supplementation with 20% alfalfa meal can increase muscle DAA and EAA contents, as well as linoleic acid and α-linolenic acid contents in PUFA, while decreasing the n-6 PUFA/n-3 PUFA ratio, which is conducive to n-3 PUFA enrichment in muscle and can improve pork quality and nutritional value.

Full Text

Effects of Alfalfa Meal on Meat Quality, Muscle Amino Acids and Fatty Acids Contents of Finishing Pigs

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Abstract: This experiment was conducted to investigate the effects of dietary alfalfa meal on growth performance, meat quality, and muscle amino acid and fatty acid contents of finishing pigs. One hundred and thirty healthy "Landrace × Yorkshire" or "Yorkshire × Landrace" crossbred finishing pigs with an average body weight of (60.28$±$0.73) kg were randomly allocated to 5 groups with 26 replicates per group and 1 pig per replicate. The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 5%, 10%, 20%, and 30% alfalfa meal, respectively. The pre-experimental period lasted 7 days, and the experimental period lasted 72 days. The results showed: 1) The 5% alfalfa meal group exhibited the highest average daily gain (ADG) and the lowest weight gain cost. 2) Dietary alfalfa meal supplementation had no significant effects on muscle pH at 45 min, cooked meat percentage, marbling score, or meat color ($P>0.05$). Compared with the control group, muscle pH at 24 h was significantly decreased in all alfalfa meal groups ($P<0.01$). Drip loss in the 30% alfalfa meal group was significantly lower than that in the control and 5% alfalfa meal groups ($P<0.05$). 3) Compared with the control group, the 20% alfalfa meal group showed significantly increased contents of delicious amino acids (DAA) (aspartic acid, glutamic acid, glycine, alanine), essential amino acids (EAA) (methionine, valine, isoleucine, phenylalanine, leucine, threonine, lysine), histidine, arginine, tyrosine, serine, proline, and total amino acids (TAA) ($P<0.05$). 4) With increasing dietary alfalfa meal levels, muscle saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) contents gradually decreased, while muscle unsaturated fatty acid (UFA), polyunsaturated fatty acid (PUFA), n-6 PUFA, and n-3 PUFA contents gradually increased. The

20% and 30% alfalfa meal groups had significantly higher muscle PUFA, linoleic acid, α-linolenic acid, n-6 PUFA, and n-3 PUFA contents compared with the control, 5%, and 10% alfalfa meal groups ($P<0.05$), and their n-6 PUFA/n-3 PUFA ratios were significantly lower ($P<0.01$). In conclusion, dietary supplementation with 20% alfalfa meal can increase muscle DAA and EAA contents, elevate linoleic acid and α-linolenic acid levels in PUFA, reduce the n-6 PUFA/n-3 PUFA ratio, and promote n-3 PUFA enrichment in muscle, thereby improving pork quality and nutritional value.

Keywords: alfalfa meal; finishing pigs; amino acids; fatty acids; n-6 PUFA/n-3 PUFA

1.1 Experimental Materials

The alfalfa meal was produced from natural drying and processing of first-bloom alfalfa from the same batch, provided by Zhenping Minxia Animal Husbandry Co., Ltd., Henan Province. Its conventional nutrient composition is shown in Table 1 .

1.2 Experimental Design and Management

The feeding trial was conducted at Henan Xinda Animal Husbandry Co., Ltd., and the slaughter trial was performed at Henan Qinyang Food Co., Ltd. One hundred and thirty healthy "Landrace × Yorkshire" or "Yorkshire × Landrace" crossbred finishing pigs with an average body weight of (60.28$±$0.73) kg were randomly divided into 5 groups with 26 replicates per group and 1 pig per replicate. The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 5%, 10%, 20%, and 30% alfalfa meal, respectively. The Osborne swine performance testing system (USA) was used for feeding. The pre-experimental period lasted 7 days, and the experimental period lasted 72 days. Diets were formulated according to NRC (2012) nutrient requirements for pigs and the actual feeding levels of the farm. The composition and nutrient levels of experimental diets are shown in Table 2 .

Pigs were fed twice daily (08:00 and 17:00) with ad libitum access to feed and water. Other management and disease prevention protocols followed standard farm practices. At the end of the feeding trial, when the average body weight reached (114.00$±$3.07) kg, 5 pigs with similar body weight were selected from each group (25 pigs total). After 24 h of fasting (with free access to water), the pigs were slaughtered. Immediately after slaughter, samples of the left carcass longissimus dorsi muscle were collected, rinsed with 4°C phosphate-buffered saline (PBS), bagged, numbered, snap-frozen in liquid nitrogen, and stored at -20°C for subsequent determination of meat quality, muscle amino acids, and fatty acids.

1.3.1 Growth Performance

The Osborne swine performance testing system (USA) was used to record daily feed intake time, feeding frequency, feed intake, initial and final body weights, and growth days at different stages. Based on these data, average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) during the 60-100 kg finishing stage were calculated.

1.3.2 Meat Quality

Meat quality parameters including pH at 45 min and 24 h (measured with a PB-10 Sartorius pH meter), meat color (using American NPPC color standards), marbling score, cooked meat percentage, and drip loss of the longissimus dorsi muscle were determined according to NY/T 821-2004 "Technical Specifications for Determination of Porcine Muscle Quality" and methods described in "Swine Production Science" [13].

1.3.3 Muscle Amino Acid Content

After thawing, longissimus dorsi muscle samples were processed according to GB/T 5009.124-2003, and amino acid contents were determined using an automatic amino acid analyzer (Hitachi L-8900).

1.3.4 Muscle Fatty Acid Content

After thawing, muscle fatty acids were extracted according to GB/T 9695.2-2008 "Meat and Meat Products—Determination of Fatty Acids" and analyzed using a gas chromatograph (Agilent 6890N).

1.4 Statistical Analysis

Experimental data were analyzed using SPSS 20.0 software for one-way ANOVA, with Duncan' s multiple range test used for inter-group comparisons. Results were expressed as "mean ± standard deviation (mean±SD)" , with $P<0.05$ considered statistically significant.

2.1 Effects of Alfalfa Meal on Growth Performance of Finishing Pigs

As shown in Table 3 , during the 60-100 kg finishing stage, dietary supplementation with 5% alfalfa meal tended to promote ADG, which was 2.24% higher than the control group, but the difference was not significant ($P>0.05$). Compared with the control group, ADFI and F/G in all alfalfa meal groups decreased, but not significantly ($P>0.05$). Weight gain cost increased with 10%, 20%, and 30% alfalfa meal supplementation, with the 30% alfalfa meal group showing a significant increase compared with the control group ($P<0.05$), while other groups showed no significant differences ($P>0.05$).

2.2 Effects of Alfalfa Meal on Meat Quality of Finishing Pigs

As shown in Table 4 , there were no significant differences in muscle pH at 45 min, cooked meat percentage, marbling score, or meat color among all groups ($P>0.05$). Compared with the control group, muscle pH at 24 h was significantly decreased in all alfalfa meal groups ($P<0.01$), but no significant differences were observed among the alfalfa meal groups ($P>0.05$). Drip loss in the control and 5% alfalfa meal groups was significantly higher than that in the 30% alfalfa meal group ($P<0.05$), but not significantly different from the 10% and 20% alfalfa meal groups ($P>0.05$).

2.3 Effects of Alfalfa Meal on Muscle Amino Acid Contents of Finishing Pigs

As shown in Table 5 , the 20% alfalfa meal group exhibited significantly higher contents of total amino acids (TAA), essential amino acids (EAA), and delicious amino acids (DAA) compared with the control, 5%, and 10% alfalfa meal groups ($P<0.05$). These values were also higher than the 30% alfalfa meal group, but the differences were not significant ($P>0.05$). No significant differences were observed among the control, 5%, 10%, and 30% alfalfa meal groups ($P>0.05$). The 20% alfalfa meal group showed significantly higher DAA contents (aspartic acid, glutamic acid, glycine, alanine) compared with the control, 5%, and 10% alfalfa meal groups ($P<0.05$), but not significantly different from the 30% alfalfa meal group ($P>0.05$). The 30% alfalfa meal group had significantly higher alanine content than the control, 5%, and 10% alfalfa meal groups ($P<0.05$). Except for tryptophan, the 20% alfalfa meal group showed significantly higher contents of the other 7 EAAs compared with the control group ($P<0.05$). The 20% alfalfa meal group also had significantly higher histidine, arginine, tyrosine, serine, and proline contents ($P<0.05$), but significantly lower cysteine content ($P<0.05$) compared with the control group. No significant differences were observed in DAA/TAA or EAA/TAA ratios among all groups ($P>0.05$).

2.4 Effects of Alfalfa Meal on Muscle Fatty Acid Contents of Finishing Pigs

As shown in Table 6 , with increasing alfalfa meal supplementation levels, muscle saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) contents gradually decreased, while muscle unsaturated fatty acid (UFA), polyunsaturated fatty acid (PUFA), n-6 PUFA, and n-3 PUFA contents gradually increased. The 30% alfalfa meal group had significantly lower muscle SFA content compared with the control and 5% alfalfa meal groups ($P<0.05$), but not significantly different from the 10% and 20% alfalfa meal groups ($P>0.05$). The 30% alfalfa meal group showed significantly lower muscle MUFA content compared with the control, 5%, and 10% alfalfa meal groups ($P<0.01$), and significantly lower than the 20% alfalfa meal group ($P<0.05$). The 20% alfalfa meal group had significantly lower muscle MUFA content compared with the control and 5% alfalfa meal groups ($P<0.01$), and significantly lower than the 10% alfalfa

meal group (P<0.05). The 30% alfalfa meal group exhibited significantly higher muscle UFA content compared with the control and 5% alfalfa meal groups (P<0.01), and significantly higher than the 10% alfalfa meal group (P<0.05), but not significantly different from the 20% alfalfa meal group (P>0.05). The 30% alfalfa meal group had significantly higher muscle PUFA, n-6 PUFA, and n-3 PUFA contents compared with the control, 5%, and 10% alfalfa meal groups (P<0.01), and significantly higher than the 20% alfalfa meal group (P<0.05). Among MUFAs, the 20% and 30% alfalfa meal groups showed significantly lower oleic acid content compared with the control group (P<0.05). Among PUFAs, the 20% and 30% alfalfa meal groups had significantly higher linoleic acid and α-linolenic acid contents compared with the control, 5%, and 10% alfalfa meal groups (P<0.05). The 30% alfalfa meal group also showed significantly higher γ-linolenic acid content compared with the control group (P<0.05). Dietary supplementation with 20% and 30% alfalfa meal significantly decreased the n-6 PUFA/n-3 PUFA ratio compared with the control, 5%, and 10% alfalfa meal groups (P<0.01), which is beneficial for n-3 PUFA enrichment in muscle.

3.1 Effects of Alfalfa Meal on Growth Performance of Finishing Pigs

Previous studies have shown that dietary alfalfa meal supplementation can improve growth performance of finishing pigs to some extent. Adding 5%-10% high-quality alfalfa meal to growing pig diets can achieve favorable growth performance and carcass traits [14-15]. Zhang et al. [16] found that adding 5%-9% alfalfa meal to 20-90 kg finishing pig diets met nutritional requirements but did not significantly affect weight gain. Xu et al. [8] reported that adding 5% and 10% alfalfa meal to crossbred pig diets improved weight gain and feed conversion efficiency, while increasing the level to 15%-20% decreased these parameters compared with the control group. Wang [11] observed that alfalfa meal supplementation had no significant effects on finishing pig growth performance, though 7% and 14% supplementation tended to improve weight gain and feed-to-gain ratio. Zhao [17] reported that 10% and 15% alfalfa meal supplementation significantly increased daily gain and decreased feed-to-gain ratio in finishing pigs. Our results showed that 5% alfalfa meal supplementation improved ADG and decreased F/G and weight gain cost, indicating that appropriate alfalfa meal levels in finishing pig diets are feasible, meeting energy requirements while improving feed utilization. However, when alfalfa meal level increased to 30%, ADG decreased and weight gain cost increased significantly, possibly due to high-fiber diets increasing satiety and reducing feed intake. It may also be related to dietary fiber levels exceeding the digestive capacity of finishing pigs, with anti-nutritional factors in fiber affecting nutrient digestion and absorption, thereby impairing growth performance [18].

3.2 Effects of Alfalfa Meal on Meat Quality of Finishing Pigs

The decline in pork quality is associated with extensive lipid peroxidation in post-slaughter muscle and cell membrane damage. Alfalfa is rich in bioactive

substances such as saponins, flavonoids, and polysaccharides, whose antioxidant effects protect muscle cell integrity and improve meat quality. Studies have shown that alfalfa meal supplementation in finishing pig diets can reduce backfat thickness and increase dressing percentage and lean meat percentage [10], with no significant effects on pH, drip loss, cooked meat percentage, meat color, or marbling, but some improvement in sensory meat quality [17,19-20]. In this experiment, alfalfa meal supplementation had no significant effects on muscle pH at 45 min, cooked meat percentage, or marbling score, but significantly decreased pH at 24 h, which remained within the normal range and may be related to increased muscle UFA content. With increasing alfalfa meal levels, drip loss gradually decreased and meat color improved, indicating that alfalfa meal supplementation can improve pork color and water-holding capacity, thereby enhancing meat quality.

3.3 Effects of Alfalfa Meal on Muscle Amino Acid Contents of Finishing Pigs

The type, content, and ratio of amino acids are related to muscle quality and flavor [21]. EAA content determines muscle protein quality, while major DAA such as glutamic acid, glycine, aspartic acid, and alanine provide the material basis for muscle flavor [22]. Studies have shown that adding appropriate levels of green forage to growing pig diets can increase muscle EAA and DAA contents and improve meat quality and flavor [23-26]. Our study found that 20% alfalfa meal supplementation significantly increased the contents of four major DAA (aspartic acid, glutamic acid, alanine, glycine) and seven human EAA (methionine, valine, isoleucine, phenylalanine, leucine, threonine, lysine), as well as TAA content. This indicates that alfalfa meal is beneficial for EAA and DAA accumulation in finishing pig muscle, with 20% supplementation significantly improving pork flavor and quality.

3.4 Effects of Alfalfa Meal on Muscle Fatty Acid Contents of Finishing Pigs

With increasing health consciousness, consumers pay more attention to meat fatty acid composition. Fatty acids, including MUFA, PUFA, and SFA, are the main components of meat fat and the basis for meat-specific flavor [27-28]. PUFA regulates hormone metabolism and enzyme activity, playing important roles in lipid metabolism regulation, cardiovascular disease prevention, and immune function delay. Studies have shown that pork fatty acid profile is affected by dietary fatty acid composition [29-30], and adding linseed oil or linseed rich in linolenic acid to finishing pig diets can significantly increase pork PUFA content [31-32]. The α-linolenic acid content in pork adipose tissue is highly correlated with dietary α-linolenic acid content [33]. Our results showed that with increasing alfalfa meal levels, muscle SFA and MUFA contents gradually decreased, while muscle UFA, PUFA, n-6 PUFA, and n-3 PUFA contents gradually increased. Dietary supplementation with 20% and 30% alfalfa meal significantly

increased muscle PUFA content, especially linoleic acid and α-linolenic acid, which may be related to the rich PUFA content in alfalfa. Forage contains abundant PUFA such as linoleic acid and linolenic acid, with alfalfa having the highest α-linolenic acid content, followed by linoleic acid, and lower contents of oleic acid and stearic acid [34]. This suggests that 20% and 30% alfalfa meal supplementation can increase n-3 long-chain fatty acid content, which is absorbed through the digestive tract and directly incorporated into carcass fat without hydrogenation [29], providing a potential approach for producing PUFA-enriched pork products.

PUFA with important biological functions are typically n-3 PUFA and n-6 PUFA. An appropriate n-6 PUFA/n-3 PUFA ratio is crucial for maintaining balance in multiple metabolic pathways [35] and serves as an important indicator for evaluating dietary fatty acid quality. Research shows that the low mortality rate from cardiovascular disease in Japanese populations is associated with a dietary n-6 PUFA/n-3 PUFA ratio of approximately 4 [36]. The Food and Agriculture Organization (FAO) recommended in 1994 that the optimal dietary n-6 PUFA/n-3 PUFA ratio for humans is 5-10 [37]. Studies have shown that increased dietary α-linolenic acid competes with γ-linolenic acid during metabolism [38], thereby increasing muscle n-3 PUFA content and significantly decreasing the n-6 PUFA/n-3 PUFA ratio [39]. In this experiment, when alfalfa meal supplementation increased to 20% and 30%, the muscle n-6 PUFA/n-3 PUFA ratio in finishing pigs was significantly decreased to 12.53 and 12.92, respectively, which is beneficial for n-3 PUFA enrichment in muscle. Although high muscle UFA content may soften muscle fat and increase susceptibility to oxidative rancidity during storage and processing, increasing muscle PUFA content is beneficial for human health. Research on the effects of alfalfa meal on muscle amino acid and fatty acid composition in finishing pigs is still in the exploratory stage. Alfalfa contains various bioactive substances such as saponins, polysaccharides, and flavonoids [2]; however, which component regulates pork amino acid and fatty acid composition and the specific mechanisms require further investigation.

4 Conclusion

In summary, dietary supplementation with 20% alfalfa meal significantly increased the contents of delicious amino acids (DAA), essential amino acids (EAA), linoleic acid, and α-linolenic acid in polyunsaturated fatty acids (PUFA), while significantly decreasing the n-6 PUFA/n-3 PUFA ratio in finishing pig muscle. This promotes n-3 PUFA enrichment in muscle, enhances pork flavor, and improves pork quality and nutritional value.

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Note: Figure translations are in progress. See original paper for figures.