

Effects of Dietary Canola Meal Level on Production Performance, Egg Quality, and Yolk Trimethylamine Content in Laying Hens with Flavin-Containing Monooxygenase 3 Genotypes: Postprint

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Abstract

This study investigated the effects of dietary canola meal levels on production performance, egg quality, and egg yolk trimethylamine (TMA) content in laying hens with different flavin-containing monooxygenase 3 (FMO3) genotypes. A total of 336 brown-shell laying hens with known FMO3 genotypes were selected, including 144 heterozygous (AT) and 144 mutant (TT) genotype hens, each randomly divided into 4 groups with 6 replicates of 6 hens each; and 48 wild-type (AA) genotype hens, randomly divided into 4 groups with 6 replicates of 2 hens each. Each group was fed experimental diets containing canola meal at inclusion levels of 0 (control), 7%, 14%, and 21% for a 6-week trial period. The results showed: 1) Dietary canola meal level, FMO3 genotype, and their interaction significantly affected the average daily feed intake of laying hens ($P < 0.05$), but had no significant effects on average egg weight or feed-to-egg ratio ($P > 0.05$); the average daily feed intake in the 14% and 21% canola meal level groups was significantly lower than that in the control group ($P < 0.05$); the AA genotype group exhibited significantly lower average daily feed intake and laying rate compared to other genotype groups ($P < 0.05$). 2) Dietary canola meal level, FMO3 genotype, and their interaction had no significant effects on egg shape index, eggshell strength, albumen height, or Haugh unit in laying hens ($P > 0.05$); however, canola meal level significantly affected egg yolk color score ($P < 0.05$), with the 21% canola meal level group showing significantly lower yolk color score than other groups ($P < 0.05$). 3) Dietary canola meal level, FMO3 genotype, and their interaction significantly affected egg yolk TMA content in laying hens ($P < 0.05$); egg yolk TMA content increased with increasing dietary canola meal inclusion levels, with the 14% and 21% canola meal level groups

showing significantly higher yolk TMA content than other groups ($P < 0.05$); the TT genotype group exhibited significantly higher yolk TMA content than the AA and AT genotype groups ($P < 0.05$). Based on the relationship between egg yolk TMA content (Y) and dietary canola meal level (X), regression equations were derived: $Y = 0.5004X + 3.2961$ ($R^2 = 0.9670$) for TT genotype; $Y = 0.0966X + 1.8054$ ($R^2 = 0.9617$) for AA genotype. To maintain yolk TMA content below the olfactory threshold, dietary canola meal level should be below 5.3% for TT genotype laying hens and below 60.1% for AA genotype laying hens. The results suggest that when feeding canola meal to laying hens, if TT genotype hens do not produce fishy-odor eggs, AA and AT genotype hens will also not produce fishy-odor eggs; when dietary canola meal level is below 5.3%, TT genotype laying hens will not produce fishy-odor eggs, and there will be no adverse effects on production performance or egg quality.

Full Text

Effects of Dietary Canola Meal Level on Performance, Egg Quality, and Trimethylamine Content in Egg Yolk of Flavin-Containing Monooxygenase 3 Genotype Laying Hens

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Abstract

This experiment investigated the effects of dietary canola meal level on production performance, egg quality, and trimethylamine (TMA) content in egg yolk of flavin-containing monooxygenase 3 (FMO3) genotype laying hens. A total of 336 laying hens with known FMO3 genotypes were used, including 144 hens each of heterozygous (AT) and mutant (TT) genotypes, and 48 hens of wild-type (AA) genotype. The AT and TT genotypes were each randomly divided into 4 groups with 6 replicates per group and 6 hens per replicate, while the AA genotype was randomly divided into 4 groups with 6 replicates per group and 2 hens per replicate. Each group was fed experimental diets supplemented with 0% (control), 7%, 14%, or 21% canola meal for a 6-week experimental period. The results showed: 1) Dietary canola meal level, FMO3 genotype, and their interaction significantly affected average daily feed intake (ADFI) ($P < 0.05$), but had no significant effects on average egg weight or feed-to-egg ratio ($P > 0.05$). The ADFI in the 14% and 21% canola meal groups was significantly lower than in the control group ($P < 0.05$). The ADFI and egg production rate in the AA genotype group were significantly lower than in other genotype groups ($P < 0.05$). 2) Dietary canola meal level, FMO3 genotype, and their interaction had no sig-

nificant effects on egg shape index, eggshell strength, albumen height, or Haugh unit ($P>0.05$). However, canola meal level significantly affected yolk color value ($P<0.05$), with the 21% canola meal group showing significantly lower yolk color values than other groups ($P<0.05$). 3) Dietary canola meal level, FMO3 genotype, and their interaction significantly affected yolk TMA content ($P<0.05$). Yolk TMA content increased with increasing dietary canola meal levels, with the 14% and 21% canola meal groups showing significantly higher TMA content than other groups ($P<0.05$). The TT genotype group exhibited significantly higher yolk TMA content than both AA and AT genotype groups ($P<0.05$). Regression equations relating yolk TMA content (Y) to dietary canola meal level (X) were derived: $Y=0.5004X+3.2961$ ($R^2=0.9670$) for TT genotype, and $Y=0.0966X+1.8054$ ($R^2=0.9617$) for AA genotype. Based on these equations, to keep yolk TMA content below the human olfactory threshold, dietary canola meal levels should be maintained below 5.3% for TT genotype hens and below 60.1% for AA genotype hens. These results suggest that when TT genotype hens do not produce fishy eggs on a canola meal diet, AA and AT genotype hens will also not produce fishy eggs. Dietary canola meal levels below 5.3% prevent fishy egg production in TT genotype hens without adversely affecting production performance or egg quality.

Keywords: canola meal; laying hens; performance; trimethylamine

Introduction

Canola meal readily induces fishy-tainted eggs because its sinapine content serves as a precursor for fishy odor formation. With the successful breeding and widespread promotion of double-low rapeseed varieties in China, the harmful components glucosinolates and oxazolidinethiones in canola meal have been substantially reduced, making sinapine a key anti-nutritional factor currently affecting the feed value of canola meal. The fishy taint problem in eggs limits the application of canola meal in layer diets, so reducing the incidence of fishy eggs is theoretically and practically important for improving egg flavor and rationally utilizing canola meal. Sinapine is a choline ester formed from choline and sinapic acid that is hydrolyzed by cecal microorganisms into choline, which is then converted to trimethylamine (TMA). Fishy taint syndrome results from mutations in the flavin-containing monooxygenase 3 (FMO3) gene that impair TMA metabolism in laying hens, causing TMA to accumulate and deposit in eggs, producing an unpleasant fishy odor. This syndrome occurs primarily in brown-shelled egg layers. Honkatukia et al. identified 17 polymorphic sites in the chicken FMO3 gene, but only the mutation at position 984 in the coding region (adenine to thymine substitution) was significantly associated with fishy taint syndrome. This mutation changes the 329th amino acid from threonine to serine.

Based on the T329S variation, individuals can be classified into three genotypes:

heterozygous (AT), mutant (TT), and wild-type (AA). Previous research has focused on genetic mutations and the effects of dietary TMA precursors on egg TMA content and mechanisms, but few reports address egg flavor issues and canola meal use in production. This study examined the effects of different dietary canola meal levels on production performance, egg quality, and yolk TMA deposition in three FMO3 genotypes of laying hens, and explored the relationship between dietary canola meal level and yolk TMA content to provide reference for rational canola meal application and egg flavor research.

Materials and Methods

1.1 Experimental Animals Healthy 47-week-old Jinghong laying hens were selected at the Pinggu Layer Experimental Base of the Feed Research Institute, Chinese Academy of Agricultural Sciences. Blood was collected from the comb for DNA extraction. The FMO3 genotype was determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method established by our research group. A total of 3,018 hens were genotyped, yielding 48 AA genotype hens, 2,556 AT genotype hens, and 414 TT genotype hens.

1.2 Experimental Design A 4\$×\$3 factorial design was employed based on canola meal level and FMO3 genotype. Three hundred thirty-six 55-week-old brown-shell laying hens with known FMO3 genotypes were used, including 144 AT genotype hens and 144 TT genotype hens. Each genotype was randomly divided into 4 groups with 6 replicates per group and 6 hens per replicate. The 48 AA genotype hens were randomly divided into 4 groups with 6 replicates per group and 2 hens per replicate. Four canola meal levels were established for each genotype: 0% (control), 7%, 14%, and 21%. The experiment included a 1-week pre-trial period followed by a 6-week experimental period.

1.3 Experimental Diets and Management Experimental diets were formulated according to NRC (1994), the “Feeding Standard of Chicken” (NY/T 33-2004), and the “Jinghong Layer Management Manual.” The control diet was a corn-soybean meal basal diet (0% canola meal). Based on the principle of equal energy and equal nitrogen, experimental diets containing 7%, 14%, and 21% canola meal were prepared. The composition and nutrient levels of experimental diets are shown in Table 1 .

All experimental hens received identical conventional management. The premix provided per kilogram of diet: VA 12,500 IU, VD₃ 4,152 IU, VE 15 IU, VK 2 mg, thiamine 1 mg, riboflavin 8.5 mg, pantothenic acid 50 mg, nicotinic acid 32.5 mg, pyridoxine 8 mg, biotin 2 mg, folic acid 5 mg, VB₁₂ 5 mg, choline 500 mg, Mn 65 mg, I 1 mg, Fe 60 mg, Cu 8 mg, Zn 66 mg, Se 0.3 mg. Sinapine and choline contents were measured values, while other nutrients were calculated values.

1.4 Measurements 1.4.1 Production Performance

During the experimental period, daily egg number and egg weight were recorded per replicate to calculate egg production rate (EP) and average egg weight (AEW). Feed remaining was weighed every two weeks to calculate average daily feed intake (ADFI) and feed-to-egg ratio (F/E) per replicate.

1.4.2 Egg Quality

On day 42, three eggs (two for AA genotype) approaching average egg weight were randomly selected from each replicate. Eggshell strength, albumen height, Haugh unit, and yolk color were measured using egg quality analyzers from ORKA (Israel). Egg shape index was measured using equipment from Fujitsu (Japan).

1.4.3 Yolk TMA Content

On day 14, three eggs (two for AA genotype) approaching average egg weight were randomly selected from each replicate. Yolks were separated and mixed. Yolk TMA content was determined using a headspace gas chromatograph from Bruker (USA).

1.5 Statistical Analysis Experimental data were analyzed using SPSS 16.0 statistical software with a GLM model for 4\$×\$3 factorial analysis. Multivariate analysis of variance was performed for main effects of genotype and canola meal level and their interaction. One-way ANOVA with Duncan' s multiple comparison was used to test differences in yolk TMA content among genotypes at the same canola meal level. Significance was declared at $P<0.05$.

Results

2.1 Effects of Dietary Canola Meal Level and FMO3 Genotype on Production Performance Statistical analysis showed no significant differences in production performance among groups during weeks 1-2, 3-4, and 5-6 (data not shown). Table 2 presents results for weeks 1-6. As shown in Table 3, dietary canola meal level, FMO3 genotype, and their interaction significantly affected ADFI ($P<0.05$), but had no significant effects on average egg weight or feed-to-egg ratio ($P>0.05$). ADFI decreased with increasing dietary canola meal levels, with the 14% and 21% canola meal groups showing significantly lower ADFI than the control group ($P<0.05$), though not significantly different from the 7% canola meal group ($P>0.05$). The AA genotype group exhibited significantly lower ADFI and egg production rate than other genotype groups ($P<0.05$).

2.2 Effects of Dietary Canola Meal Level and FMO3 Genotype on Egg Quality The effects of dietary canola meal level and FMO3 genotype on egg quality are shown in Table 2. Multivariate analysis (Table 3) revealed that canola meal level, FMO3 genotype, and their interaction had no significant effects on egg shape index, eggshell strength, albumen height, or Haugh unit ($P>0.05$). However, canola meal level significantly affected yolk color value

($P < 0.05$). Yolk color value decreased with increasing dietary canola meal levels, with the 21% canola meal group showing significantly lower values than other groups ($P < 0.05$).

2.3 Effects of Dietary Canola Meal Level and FMO3 Genotype on Yolk TMA Content As shown in Tables 2 and 3, dietary canola meal level, FMO3 genotype, and their interaction significantly affected yolk TMA content ($P < 0.05$). Yolk TMA content increased with increasing dietary canola meal levels. Main effect analysis (Table 3) showed that compared with the control group, the 7%, 14%, and 21% canola meal groups had significantly elevated yolk TMA content ($P < 0.05$), with the 14% (6.80 g/g) and 21% (8.01 g/g) canola meal groups significantly higher than the 7% canola meal group (5.23 g/g) ($P < 0.05$). For genotype, the TT genotype group showed significantly higher yolk TMA content than AA and AT genotype groups ($P < 0.05$), with the AA genotype group having the lowest TMA content. The interaction between dietary canola meal level and FMO3 genotype was evident: no significant differences among genotypes were observed in the control group ($P > 0.05$); in the 7% and 14% canola meal groups, the TT genotype group was significantly higher than AA and AT groups ($P < 0.05$); in the 21% canola meal group, the TT genotype group was significantly higher than the AT group, which was significantly higher than the AA group ($P < 0.05$).

Additionally, significant linear correlations were observed between yolk TMA content and dietary canola meal level for TT and AA genotypes ($P < 0.05$). For TT genotype hens, the relationship between yolk TMA content (Y) and dietary canola meal level (X) was: $Y = 0.5004X + 3.2961$ ($R^2 = 0.9670$). For AA genotype hens, the relationship was: $Y = 0.0966X + 1.8054$ ($R^2 = 0.9617$). Based on the olfactory threshold of 4 g/g for yolk TMA content, dietary canola meal levels should be maintained below 5.3% for TT genotype hens and below 60.1% for AA genotype hens to prevent detection of fishy odor. In this experiment, even at 21% dietary canola meal, the AA genotype group did not exceed the olfactory threshold.

Discussion

3.1 Effects of Dietary Canola Meal Level and FMO3 Genotype on Production Performance This study demonstrated that dietary canola meal level, FMO3 genotype, and their interaction had no significant effects on average egg weight or feed-to-egg ratio, but significantly affected ADFI. Previous research indicates that diets containing 20% low-glucosinolate canola meal do not affect animal performance, consistent with our results. However, ADFI decreased with increasing dietary canola meal levels in this study, with the 14% and 21% canola meal groups showing significantly lower ADFI than the control. Richter et al. found that diets containing 20% canola meal significantly reduced ADFI in laying hens. Phenolic compounds are the primary cause of dark color, bitterness, and astringency in canola meal and likely contribute to

reduced ADFI. The main phenolics in canola meal are sinapine and tannins. Sinapine is a bitter-tasting sinapic acid choline ester and a major contributor to canola meal bitterness. Ismail et al. reported that 50-91% of the bitterness in canola meal aqueous suspensions originates from sinapine and free choline. Studies have shown that diets containing 2% sinapine thiocyanate significantly reduced feed intake in mice. Tannin content in canola meal ranges from 1.5% to 3%, imparting a pungent and bitter taste that affects palatability and may reduce feed intake.

Few reports exist on the effects of FMO3 genotype on layer performance. Rapid FMO3 genotyping and elimination of mutant genotypes have been widely applied in layer breeding programs. Settar et al. reported that FMO3 genotype was significantly associated with age at sexual maturity, shell color, and egg weight. In Rhode Island Red layers, egg weight was significantly affected by FMO3 genotype, with AA genotype showing the lowest egg weight. Due to the unbalanced distribution of genotypes, the natural frequency of AA genotype is very low in commercial layer populations, resulting in limited AA individuals for this experiment. Further research is needed to confirm the effects of genotype on production performance.

3.2 Effects of Dietary Canola Meal Level and FMO3 Genotype on Egg Quality

Dietary canola meal level and FMO3 genotype had no significant effects on egg shape index, eggshell strength, albumen height, or Haugh unit, consistent with Kretzschmar et al. Our previous research also showed that FMO3 genotype had no significant effects on conventional egg quality traits, moisture content, crude protein and fat content, or yolk ratio. However, canola meal level significantly affected yolk color value, which decreased with increasing dietary canola meal levels. Riyazi et al. found that the 15% canola meal group had lower yolk color values than the 5%, 10%, and control groups. Poultry cannot synthesize pigments, and only oxygen-functionalized carotenoids (hydroxy, acyl, and keto groups) including lutein, lycopene, and zeaxanthin have coloring effects. Dietary fat type, quality, quantity, and oxidation status affect pigment deposition. Oxidized carotenoids are lipophilic substances, and low dietary fat levels can impair their absorption, cause feed deterioration, and reduce bile secretion, significantly decreasing carotenoid absorption, transport, and deposition. High canola meal levels contain substantial anti-nutritional factors including sinapine and tannins. Research indicates that sinapine significantly inhibits lipase and lipoxygenase activities. High canola meal diets may reduce yolk color values by suppressing lipase activity, thereby affecting dietary fat characteristics and carotenoid utilization. Other anti-nutritional factors may also directly or indirectly reduce yolk color values. Another major reason may be the reduced corn percentage in diet formulations as canola meal levels increase.

3.3 Effects of Dietary Canola Meal Level and FMO3 Genotype on Yolk TMA Content

Feeding canola meal readily induces fishy-tainted eggs, with fishy eggs detectable within 5 days of feeding canola meal-containing di-

ets. Removing canola meal from diets eliminates fishy egg production. Sinapine in canola meal serves as the precursor for the fishy compound TMA. TMA metabolism in laying hens is influenced by both genetic and nutritional factors, with individual genetic background (FMO3 genotype) and dietary precursor levels being two critical factors affecting egg TMA content. This study demonstrated that dietary canola meal level, FMO3 genotype, and their interaction significantly affected yolk TMA content. Yolk TMA content increased significantly with dietary canola meal levels, and increasing canola meal levels significantly elevated yolk TMA content in TT genotype hens. Previous research suggests that susceptible genotype (TT) hens show a significant linear relationship between egg TMA content and canola meal level, while AA and AT genotypes show no increasing trend. However, this study observed a linear relationship between yolk TMA content and dietary canola meal level in AA genotype hens, though even at 21% canola meal, AA genotype hens did not exceed the olfactory threshold. Additionally, at 14% dietary canola meal, AT and AA genotypes showed significantly different yolk TMA contents, consistent with Kretzschmar et al. This may be because fishy egg production exhibits additive or semi-dominant inheritance when feeding high canola meal levels.

The human olfactory threshold for whole egg TMA is 1 g/g. Ward et al. inferred a yolk TMA threshold of 4 g/g based on yolk proportion. As TMA content increases, fishy odor intensifies, flavor scores decrease, and consumer acceptance declines. Adding 3% conventional canola meal can cause fishy eggs, while the maximum canola meal level for fishy egg-free production is 4-7%. Based on the regression equation for TT genotype hens, the canola meal level corresponding to the olfactory threshold was 5.3%, which falls within Ward et al.'s reported range of 4-7%.

Conclusions

1. Dietary canola meal level and FMO3 genotype had no significant effects on average egg weight, feed-to-egg ratio, egg shape index, eggshell strength, albumen height, or Haugh unit. Average daily feed intake decreased when canola meal levels exceeded 7%, and yolk color values decreased when levels exceeded 14%.
2. Yolk TMA content in TT genotype hens reached the olfactory threshold when dietary canola meal levels exceeded 5.3%.
3. Under the conditions of this experiment, maintaining dietary canola meal levels below 5.3% effectively reduced the incidence of fishy eggs without adversely affecting production performance or egg quality.

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