Effects of Dietary Rapeseed Meal Level on Production Performance, Egg Quality, and Yolk Trimethylamine Content in Laying Hens with Flavin-Containing Monooxygenase 3 Genotype (Postprint)

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Abstract

This study investigated the effects of dietary rapeseed meal level 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 per group and 6 hens per replicate; and 48 wild-type (AA) genotype hens, randomly divided into 4 groups with 6 replicates per group and 2 hens per replicate. Each group was fed experimental diets containing 0% (control), 7%, 14%, and 21% rapeseed meal, respectively, for a 6-week experimental period. The results showed: 1) Dietary rapeseed 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 and feed-to-egg ratio ($P>0.05$); the average daily feed intake of the 14% and 21% rapeseed meal level groups was significantly lower than that of the control group ($P<0.05$); the average daily feed intake and laying rate of the AA genotype group were significantly lower than those of other genotype groups ($P<0.05$). 2) Dietary rapeseed meal level, FMO3 genotype, and their interaction had no significant effects on egg shape index, eggshell strength, albumen height, and Haugh unit of laying hens ($P>0.05$); however, rapeseed meal level significantly affected egg yolk color score ($P<0.05$), with the 21% rapeseed meal level group having significantly lower egg yolk color score than the control and 7% rapeseed meal level groups ($P<0.05$). 3) Dietary rapeseed meal level, FMO3 genotype, and their interaction significantly affected

egg yolk TMA content (P<0.05); egg yolk TMA content increased with increasing dietary rapeseed meal level, with the 14% and 21% rapeseed meal level groups having significantly higher egg yolk TMA content than other groups (P<0.05); the TT genotype group had significantly higher egg 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 rapeseed meal level (X), regression equations were obtained: $Y = 0.5004X + 3.2961$ ($R^2 = 0.967$) (TT genotype); $Y = 0.0966X + 1.8054$ ($R^2 = 0.9617$) (AA genotype). To keep egg yolk TMA content below the olfactory threshold, dietary rapeseed meal level should be below 5.3%. The results suggest that when dietary rapeseed meal level is below 5.3% in laying hens, fishy-odor eggs will not be produced, and there will be no adverse effects on production performance and 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 laying hens with different flavin-containing monooxygenase 3 (FMO3) genotypes. 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 containing 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 (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 groups was significantly lower than in the control group (P<0.05). The AA genotype group exhibited significantly lower average daily feed intake and egg production 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 (P>0.05). However, dietary canola meal level significantly affected yolk color value (P<0.05), with the 21% canola meal group showing significantly lower yolk color values than the control and 7% canola meal 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 the 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=0.967) for TT genotype, and Y=0.0966X+1.8054 (R^2=0.9617) for AA genotype. To maintain yolk TMA content below the human olfactory threshold, dietary canola meal level should be kept below 5.3%. These results suggest that dietary canola meal levels below 5.3% can effectively prevent the production of fishy-tainted eggs 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 canola varieties in China, the harmful components isothiocyanate and oxazolidinethione in canola meal have been substantially reduced, making sinapine a key antinutritional factor affecting the feed value of canola meal. The fishy taint problem limits canola meal application in layer diets, so reducing the incidence of fishy-tainted eggs is theoretically and practically important for improving egg flavor and rationalizing canola meal use. Sinapine is a choline ester formed from choline and sinapic acid that is hydrolyzed by cecal microorganisms into choline, which is subsequently converted to trimethylamine (TMA). Fishy taint syndrome results from FMO3 gene mutations in laying hens that impair TMA metabolism, leading to TMA accumulation and deposition in eggs, which produces an unpleasant fishy odor. This syndrome occurs predominantly in brown-shelled egg layers. Honkatukia et al. identified 17 polymorphic sites in the chicken FMO3 gene, with only the mutation at position 984 in the coding region (adenine to thymine substitution) being significantly associated with fishy taint syndrome. This mutation causes threonine to be replaced by serine at amino acid position 329 in the FMO3 protein. Based on this 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 dietary TMA precursor levels, but few studies have addressed egg flavor issues and canola meal use in production. This experiment examined production performance, egg quality, and yolk TMA deposition in three FMO3 genotypes of laying hens fed different canola meal

levels, 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

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. DNA was extracted from blood samples collected from the comb. The FMO3 genotype was determined using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method established by our research group. Among 3,018 hens genotyped, 48 were AA genotype, 2,556 were AT genotype, and 414 were TT genotype.

Experimental Design

A 4×3 factorial design was employed with four canola meal levels and three FMO3 genotypes. A total of 336 brown-shelled laying hens with known FMO3 genotypes were selected at 55 weeks of age, including 144 hens each of AT and TT genotypes, and 48 hens of 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 genotype received four dietary canola meal levels: 0%, 7%, 14%, and 21%. The experiment included a 1-week pre-trial period and a 6-week experimental period.

Experimental Diets and Management

Experimental diets were formulated according to NRC (1994), "Feeding Standard of Chickens" (NY/T 33-2004), and the "Jinghong Layer Management Manual." The control diet was a corn-soybean meal-based 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 hens received identical conventional management.

Table 1 Composition and nutrient levels of experimental diets (air-dry basis) %

Items	Canola meal level/%
Ingredients	
Corn	
Soybean meal	

Items	Canola meal level/%
Double-low canola meal	
Soybean oil	
CaHPO	
Limestone	
NaCl	
DL-Met	
L-Lys • HCl	
Premix[1])	
Phytase	
Zeolite powder	
Total	
Nutrient levels[2])	
ME/(MJ/kg)	
CP	
Ca	
TP	
AP	
Lys	
Met	
Sinapine/(mg/g)	
Choline	

[1]) Premix provided the following per kg of diets: VA 12,500 IU, VD 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, VB 5 mg, choline 500 mg, Mn 65 mg, I 1 mg, Fe 60 mg, Cu 8 mg, Zn 66 mg, Se 0.3 mg.

[2]) Sinapine and choline content were measured values, while the others were calculated values.

Measurements

Production Performance During the experiment, daily egg number and egg weight were recorded by replicate to calculate egg production (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) by replicate.

Egg Quality On day 42 of the experiment, three eggs per replicate (two eggs for AA genotype) with weights close to the average were randomly selected. Egg shell strength, albumen height, Haugh unit, and yolk color were measured using an ORKA egg quality analyzer (Israel). Egg shape index was measured using a Fuji Tsubo egg shape index analyzer (Japan).

Yolk TMA Content On day 14 of the experiment, three eggs per replicate (two eggs for AA genotype) with weights close to the average were randomly selected. Yolks were separated and mixed. Yolk TMA content was determined by headspace gas chromatography using a Bruker instrument (USA).

Data Processing

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. Differences in yolk TMA content among genotypes at the same canola meal level were tested using one-way ANOVA with Duncan' s multiple comparison. Significance was declared at $P<0.05$.

Results

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 average daily feed intake ($P<0.05$), but had no significant effects on average egg weight or feed-to-egg ratio ($P>0.05$). Average daily feed intake decreased with increasing dietary canola meal level; the 14% and 21% canola meal groups showed significantly lower intake than the control group ($P<0.05$), but did not differ significantly from the 7% canola meal group ($P>0.05$). The AA genotype group exhibited significantly lower average daily feed intake and egg production rate than other genotype groups ($P<0.05$).

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 level; the 21% canola meal group showed significantly lower yolk color values than the control and 7% canola meal groups ($P<0.05$).

Table 2 Effects of FMO3 genotype and dietary canola meal level on performance and egg quality of laying hens

Genotype	Canola meal level/%	ADFI/g	AEW/g	F/E	EP/%	Egg shape index	Eggshell strength/N	Albumen height/mm	Haugh unit	Egg yolk color
	0									5.00a
	7									4.36a
	14									4.44a
	21									4.11ab
	0									4.25a
	7									4.47a
	14									4.06ab
	21									4.19a
	0									4.42a
	7									4.50a
	14									3.97ab
	21									3.67b

In the same row, values with different small letter superscripts mean significant difference (P<0.05). The same as below.

Table 3 Main effects analysis of FMO3 genotype and dietary canola meal level on performance and egg quality of laying hens

Items	Means of main effects	Source of variation (P-value)
	Genotype	Canola meal level/%
ADFI/g	94.33b	0.63b
AEW/g	0.78a	0.74a
F/E	4.61a	0.73a
EP/%		
Egg shape index		
Eggshell strength/N		
Albumen height/mm		
Haugh unit		
Egg yolk color		

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

As shown in Table 4 , dietary canola meal level, FMO3 genotype, and their interaction significantly affected yolk TMA content (P<0.05). Yolk TMA content increased with increasing canola meal level. Main effect analysis revealed that compared with the control group, supplementation with 7%, 14%, and 21% canola meal significantly increased yolk TMA content (P<0.05). The 14% canola meal group (6.80 g/g) and 21% canola meal group (8.01 g/g) showed significantly higher TMA content than the 7% canola meal group (5.23 g/g)

(P<0.05). Regarding genotype, the TT genotype group exhibited significantly higher yolk TMA content than the AA and AT genotype groups (P<0.05), with the AA genotype group showing the lowest TMA content. The interaction between dietary canola meal level and FMO3 genotype was evident: no significant differences among genotypes were observed when feeding the control diet (P>0.05); when feeding 7% and 21% canola meal diets, the TT genotype group showed significantly higher TMA content than the AA and AT genotype groups (P<0.05); when feeding the 14% canola meal diet, both TT and AT genotype groups showed significantly higher TMA content than the AA genotype group (P<0.05).

Additionally, significant linear correlations were observed between yolk TMA content and canola meal level for TT and AA genotypes (P<0.05), but not for AT genotype. For TT genotype laying 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 laying 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, the calculated dietary canola meal level should be below 5.3% to prevent sensory detection of TMA in TT genotype laying hens. In this experiment, even at 21% dietary canola meal supplementation, the AA genotype group did not exceed the olfactory threshold.

Table 4 Effects of FMO3 genotype and dietary canola meal level on TMA content in egg yolk of laying hens

Canola meal level/%	Genotypes	TMA content in egg yolk/(g/g)	Pooled SEM	Means of main effects
	AA	2.57c		
	AT	3.35c		
	TT	1.97c		
	AA	8.00b		
	AT	4.80c		
	TT	2.25c		
	AA	10.08b		
	AT	7.83b		
	TT	3.13c		
	AA	13.55a		
	AT	4.87c		
	TT	3.93c		
Genotype				
AA		2.82c		
AT		5.21b		
TT		8.55a		
Canola meal level/%				
0		2.68c		

Canola meal level/%	Genotypes	TMA content in egg yolk/(g/g)	Pooled SEM	Means of main effects
7		5.23b		
14		6.80a		
21		8.01a		
Source of variation (P-value)				
Genotype				<0.05
Canola meal level				<0.05
Genotype×canola meal level				<0.05

Discussion

Effects of Dietary Canola Meal Level and FMO3 Genotype on Production Performance

This experiment 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 average daily feed intake. Previous studies reported that diets containing 20% low-glucosinolate canola meal did not affect production performance, consistent with our results. However, in this experiment, average daily feed intake decreased with increasing dietary canola meal level, with the 14% and 21% canola meal groups showing significantly lower intake than the control group. Richter et al. found that diets containing 20% canola meal significantly reduced daily feed intake. Phenolic compounds are the primary cause of the dark color, bitterness, and astringency of canola meal and may be the main factor affecting feed intake. Phenolics in canola meal mainly include sinapine and tannins. Sinapine is a bitter-tasting sinapic acid choline ester and a major source of bitterness in canola meal. Ismail et al. found that 50%-91% of the bitterness in canola meal aqueous suspensions was produced by sinapine and free choline. Studies have shown that diets containing 2% sinapine thiocyanate significantly reduced feed intake in mice. Tannin content in canola meal is approximately 1.5%-3%, imparting a pungent and bitter taste that affects palatability and also contributes to reduced feed intake in laying hens. Currently, few reports exist on the effects of FMO3 genotype on layer production performance. Rapid detection of FMO3 genotype and elimination of mutant genotypes have been widely applied in layer breeding programs worldwide. Settar et al. reported that FMO3 genotype was significantly associated with age at sexual maturity, eggshell color, and egg weight. In Rhode Island Red laying hens, egg weight was significantly affected by FMO3 genotype, with

AA genotype showing the lowest egg weight. Due to unbalanced genotype distribution, the natural occurrence of AA genotype is very low in commercial layer populations, resulting in few AA individuals in this experiment. Therefore, the effects of genotype on production performance indicators require further research and verification.

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 the findings of 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 proportion. However, canola meal level significantly affected yolk color, with yolk color becoming lighter as canola meal level increased. Riyazi et al. found that the 15% canola meal group showed significantly lighter yolk color than the 5%, 10% canola meal groups and the control group. Poultry cannot synthesize pigments; only oxygen-functional group-containing carotenoids (including lutein, lycopene, and zeaxanthin) have coloring effects. The type, quality, quantity, and oxidation status of dietary fat affect pigment deposition. Oxidized carotenoids are lipophilic substances; low dietary fat levels can impair their absorption and cause feed spoilage, and can also reduce bile secretion, significantly decreasing carotenoid absorption, transport, and deposition. High-level canola meal supplementation introduces more antinutritional factors such as sinapine and tannins. Research has shown that sinapine significantly inhibits lipase and lipoxygenase activity in vivo. High-level canola meal diets may reduce yolk color values by inhibiting lipase activity, thereby affecting dietary fat characteristics. Other antinutritional factors may also cause lighter yolk color through direct or indirect mechanisms. Another major reason may be the reduced corn percentage in diet formulation as canola meal level increases.

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

Feeding canola cake or meal readily induces fishy-tainted eggs, with fishy taint detectable within 5 days of feeding canola-containing diets. Removing canola cake or meal from the diet stops fishy egg production. Sinapine in canola cake or meal serves as a precursor for the fishy-tainting substance TMA. TMA metabolism in laying hens is influenced by both genetic and nutritional factors, with individual genetic background (FMO3 genotype) and dietary precursor content being two critical factors affecting egg TMA content. In this experiment, dietary canola meal level, FMO3 genotype, and their interaction significantly affected yolk TMA content. Yolk TMA content increased significantly with increasing canola meal level, and dietary canola meal supplementation significantly increased yolk TMA content in the TT genotype group. Research

suggests a significant linear relationship between egg TMA content and double-low canola cake supplementation level in susceptible genotypes (TT genotype), with TMA content increasing as canola cake level increases, while AA and AT genotypes show no increasing trend. In this experiment, a linear relationship between yolk TMA content and dietary canola meal level was also observed in AA genotype laying hens. However, even at 21% dietary canola meal, the AA genotype group did not exceed the olfactory threshold. Additionally, when feeding 14% canola meal, AT and AA genotypes differed significantly in yolk TMA content, consistent with Kretzschmar et al. This may be because fishy taint exhibits additive or semi-dominant inheritance when feeding high levels of canola meal.

The human olfactory threshold for TMA in whole eggs is 1 g/g. Ward et al. inferred the yolk TMA threshold to be 4 g/g based on yolk proportion in whole eggs. As TMA content increases, fishy odor intensifies, flavor scores decrease, and consumer acceptance declines. Regular canola cake supplementation at only 3% can cause fishy taint in eggs, while the maximum supplementation level of double-low canola cake without producing fishy eggs is 4%-7%. In this experiment, based on the regression equation for TT genotype laying hens, the canola meal level corresponding to the olfactory threshold was 5.3%, which falls within the 4%-7% range reported by Ward et al.

Conclusions

This study demonstrated that 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. Canola meal levels above 7% reduced feed intake, while levels above 14% lightened yolk color. Yolk TMA content in TT genotype laying hens reached the olfactory threshold when dietary canola meal level exceeded 5.3%. Under the conditions of this experiment, dietary canola meal levels below 5.3% can effectively reduce the incidence of fishy-tainted eggs without adversely affecting production performance or egg quality.

References

[1] He J. Study on extraction, separation, purification, identification and enzymatic hydrolysis of sinapine from canola meal[D]. Master' s Thesis. Wuhan: Huazhong Agricultural University, 2010.

[2] Hobson-Frohock A, Land D G, Griffiths N M, et al. Egg taints: association with trimethylamine[J]. Nature, 1973, 243(5405): 304–305.

[3] Goh Y K, Mueller M M, Clandinin D R, et al. The effects of choline and

sinapine bisulfate in a laying ration on the incidence of fishy odor in eggs from brown-shelled egg layers[J]. Canadian Veterinary Journal La Revue Veterinaire Canadienne, 1979, 59(3): 545–549.

[4] Butler E J, Pearson A W, Greenwood N M. Trimethylamine taint in eggs: the occurrence of the causative metabolic defect in commercial hybrids and pure breeds in relation shell colour[J]. Journal Science Agriculture, 1984, 35(3): 272–278.

[5] Bain M A, Fornasini G, Evans A M. Trimethylamine: Metabolic, pharmacokinetic and safety aspects[J]. Current Drug Metabolism, 2005, 6(3): 227–240.

[6] Honkatukia M, Reese K, Preisinger R. Fishy taint in chicken eggs is associated with a substitution within a conserved motif of the FMO3 gene[J]. Genomics, 2005, 86(2): 225–232.

[7] Wang J, Wu S G, Zhang H J, et al. Frequency distribution of flavin-containing monooxygenase 3 genotype and its effect on egg quality in Hy-Line brown layers[J]. Chinese Journal of Animal Nutrition, 2014, 26(3): 630–636.

[8] Ward A K, Classen H L, Buchanan F C. Fishy-egg tainting is recessively inherited when brown-shelled layers are fed Canola meal[J]. Poultry Science, 2009, 88(4): 714–721.

[9] Aherne F X, Lewis A J, Hardin R T. An evaluation of faba beans (Vicia faba) as a protein supplement for swine[J]. Canadian Journal of Animal Science, 1977, 57(2): 321–328.

[10] Richter G, Lemsner A, Bargholz J. Canola and Canola meal as components in diets of laying hens[J]. Archives of Animal Nutrition, 1996, 49(3): 229–241.

[11] Ismail F, Vaisey-Genser M, Fyfe B. Bitterness and astringency of sinapine and its components[J]. Journal of Food Science, 1981, 46(4): 1241–1244.

[12] Josefsson E, Uppström B. Influence of sinapine and p-hydroxybenzylglucosinolate on the nutritional value of Canola and white mustard meals[J]. Journal of the Science of Food and Agriculture, 1976, 27(5): 438–442.

[13] Settar P, Fulton J E, Arango J, et al. Association of FMO3 gene with egg production and quality traits in brown layers[C]//Poultry science association annual. Dallas Center, IA: Hy-Line International, 2008, 87: 70.

[14] Kretzschmar K, Danicke S, Schmutz M, et al. Interactions of flavin containing monooxygenase 3 (FMO3) genotype and feeding of choline and Canola cake on the trimethylamine (TMA) content in egg yolks of laying hens[J]. Archives of Animal Nutrition, 2009, 63(3): 173–187.

[15] Kretzschmar K, Reese K, Honkatukia M, et al. Effect of flavin containing monooxygenase (FMO3) genotype on trimethylamine (TMA) content in the chicken egg yolk[J]. Archiv für Geflügelkunde, 2007, 71(5): 200–206.

[16] Riyazi S R, Ebrahimnezhad Y, Nazeradl K, et al. The effects of replacing soybean meal with different levels of Canola meal on egg quality characteristics of commercial laying hens[J]. Asian Journal of Animal and Veterinary Advances, 2009, 4(6): 337-341.

[17] Ward A K. Genetic and dietary interactions of fishy-egg taint in brown-shelled laying hens[D]. Master' s Thesis. Saskatchewan, Saskatoon, Canada: University of Saskatchewan Saskatoon, 2008.

[18] Griffiths N M, Land D G, Hobson-Frohock A. Trimethylamine and egg taint[J]. British Poultry Science, 1979, 20(6): 555-558.

[19] Ven Dänicke S, Ueberschär K H, Reese K, et al. Investigations on the effects of rape oil quality, choline and methionine concentration in diets for laying hens on the trimethylamine content of the eggs, on trimethylamine metabolism and on laying performance[J]. Archives of Animal Nutrition, 2006, 60(1): 57-79.

[20] Wang J, Wu S G, Zhang H J, et al. Trimethylamine deposition in the egg yolk from laying hens with different FMO3 genotypes[J]. Poultry Science, 2013, 92(3): 746-752.

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