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Determination of the Effect of Trimethylamine Reduction in Egg Yolk Following Supplementation of Laying-Hen Feed with Riboflavin

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ABSTRACT - The intensity of fishy odor in eggs, which differs depending on the poultry type and individual perception, can be due to many factors including trimethylamine (TMA) which has been identified as the main. Notably, riboflavin can increase the activity of flavin-containing monooxygenase 3, the enzyme responsible for converting TMA into odorless trimethylamine-*N*-oxide. This study aimed to analyze the TMA content in egg yolk, evaluate its contribution to fishy odor, and develop a method to prevent this undesired odor. Solid-phase microextraction-gas chromatography/mass spectrometry was used to detect and quantify volatile compounds in egg yolk from hens fed a standard TMA-rich diet and hens fed a riboflavin-supplemented diet. To compare the relative content of volatile substances between eggs, a correlation study was performed using an electronic nose. Higher concentration of TMA (1.06 ± 0.12 mg/kg) was detected in egg yolks obtained from hens fed a normal diet than those fed a riboflavin-supplemented diet. Overall, this study suggests that riboflavin affects the quantity and quality of volatile substances, including TMA, present in eggs and we expect these findings to improve the quality and reduce the fishy odor of eggs.

Key words: Egg yolk, Poultry, Riboflavin, SPME-GC/MS, Trimethylamine

Eggs are the most common poultry product distributed worldwide. According to data from the Food and Agriculture Organization of the United Nations, total egg production and consumption have steadily increased globally since the beginning of the 20th century[1]. Eggs contain approximately 75% water, 11% fat, 11% protein, and 1% carbohydrate, as well as albumin, which helps in cell growth and recovery from fatigue. Eggs contain almost all the known amino acids; therefore, they are considered a complete food[2]. However, a fishy smell, often repelling to customers, usually emanates from eggs, thus affecting the egg industry. According to previous studies, the fishy taste-related properties of eggs are attributed to trimethylamine (TMA) levels in egg yolk[3].

Fishy odor in chicken and eggs was first reported in 1933, followed by follow-up studies in 1948[4]. In 1973, TMA was confirmed to be the causative substance of the fishy odor in eggs by gas chromatography (GC)[5]. Since then, TMA studies have been conducted using electronic nose, headspace, and solid-phase microextraction (SPME)-GC[6-8]. In addition to TMA, eggs contain various aromatic compounds, such as alcohols, aldehydes, ketones, esters, acids, and reduced sulfur compounds; nevertheless, TMA is believed to be the main compound responsible for their fishy odor[9].

Choline, carnitine, and glycine betaine contained in poultry feeds, which are absorbed by the hen and converted into TMA by *Enterobacteriaceae* bacteria, are believed to be responsible of the TMA that accumulates in the egg yolk[10]. Poultry feeds mainly contain choline chloride in the form of the alkaloid amine sinapine, which is an ester of choline and sinapic acid[11], that is present in some seeds. Thus, hens susceptible to sinapine are more likely to produce eggs with a fishy odor[12]. Generally, flavin-containing monooxygenase 3 (FMO3) converts TMA into trimethylamine-*N*-oxide (TMAO), which is then excreted. Goitrin, an antinutritive factor formed by the action of myrosinase on dietary glucosinolates, inhibits the oxidation of TMA into TMAO by competing for the active site of FMO3[13,14]. However, due to decreased

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activation of FMO3 or competition for its active site, TMA accumulates in the egg yolk, resulting in a fishy odor[5]. A similar event has been reported in humans, described as fish odor syndrome or trimethylaminuria. Even in a clean environment, a fishy smell can be generated by the body as TMA is excreted through the urine, sweat, and breathing[15].

Several methods focusing on diet restrictions and/or supplementation have been explored to manage the undesired effects of TMA accumulation, including restriction of choline intake, reducing intestinal bacteria using antibiotics (such as neomycin or metronidazole), and increasing FMO3 enzyme activity through riboflavin supplementation[16,17]. Restricting choline in poultry feed can affect productivity, whereas antibiotic indiscriminate use can affect egg quality[18]. Thus, this study aimed to determine the difference in TMA content between eggs produced from hens fed with normal feed and those supplemented with riboflavin. Moreover, we aimed to determine whether using riboflavin supplementation to hens could change the flavor profile induced by trimethylaminuria[17]. A total of 100,000 hens were fed riboflavin-supplemented diets for 1–5 weeks, and 60 eggs were randomly collected and analyzed to determine whether TMA was reduced or not. In the present study, TMA was detected using solid-phase microextraction–gas chromatography/mass spectrometry (SPME-GC/MS), and its levels were quantified in the egg yolk. Moreover, the effect of volatile compounds on fishy odor was evaluated using an electronic nose.

We aimed to detect and quantify TMA in egg yolk. Moreover, we aimed to evaluate the effect of volatile compounds on fishy odor. The findings of this study are expected to help identify the flavor features of eggs and provide a reference for future investigations to develop new technologies for odor analysis.

Materials and Methods

Sampling

Eggs were collected 60 days after feeding laying hens raised in the Chungcheongbuk-do region of South Korea on a diet containing choline chloride (TMA precursor) to identify compounds contributing to their fishy odor. From October 2020 to August 2021, a total of 100,000 hens were fed a riboflavin (40 mg/kg)-supplemented feed for 1–5 weeks repeatedly, and 60 eggs were randomly collected. This study was conducted according to the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines and was approved by the ethics review committee of our institute.

Analytical standards and reagents

Standard TMA (40% solution in water), trichloroacetic acid (TCA) (BioUltra ≥ 99.5%), and potassium hydroxide solution (45% [w/v] in water) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water (18.2 MΩ) was obtained using a Milli-Q gradient water system (Millipore, Bedford, MA, USA).

Preparation of sample and working solutions

SPME-GC/MS and electronic nose analyses were conducted according to the Commission Regulation (EC) No. 2074/2005, and previously described methods[6-8]. Briefly, 10 g of egg yolk was weighed and placed in a 50 mL tube. Next, 10% TCA solution was up to 30 mL final volume. The mixture was thoroughly vortexed and centrifuged at 4,500 rpm for 10 min. The supernatant was transferred to a 10 mL tube, and distilled water (5 mL) and 45% potassium hydroxide solution (2 mL) were added. Next, 2 mL of the supernatant was placed in a headspace vial and immediately sealed with a headspace cap; the solution was then thoroughly vortexed. Subsequently, SPME-GC/MS and electronic nose analyses were performed as described below.

Control egg yolk (without TMA) and water samples were spiked with increasing concentrations of TMA (0.01, 0.05, 0.1, 0.5, 1, 2, 4, and 6 mg/kg) and pretreated as described above. These samples were used to design a calibration curve for quantification of the experimental samples.

SPME-GC/MS instrumentation conditions

GC/MS analysis was performed using an Agilent 7890 Gas Chromatograph, Agilent 5975C TAD Series GC/MSD System, equipped with a DB-WAX column (60 m × 0.25 mm × 0.25 μm), and Agilent GC Sampler 80 G6501B CTC PAL Gas Autosampler (Agilent Technologies, Santa Clara, CA, USA). The sample was transferred to a headspace vial, heated, and shaken at 500 rpm for 10 min at 50°C for headspace equilibration. Then a 24 Ga, 65 μm, polydimethylsiloxane/divinylbenzene (PDMS/DVB) SPME fiber (Supelco, Bellefonte, PA, USA) was inserted into the headspace for volatile adsorption. The extraction time and

temperature were set to 30 min and 50°C, respectively. Once the volatile compounds were extracted, they were desorbed at 250°C for 1 min. After each injection, the SPME fiber was heated to 300°C under helium flow for 10 min. The liner used was an Inlet Liner, Direct SPME Type (Supelco). The splitless injection method and the DB-WAX capillary column was used under the following conditions: the oven temperature was initially set at 40°C for 5 min, increased to 60°C at a rate of 1°C/min and maintained for 5 min, and then increased to 120°C at 10°C/min and maintained for 10 min. To avoid condensation, the interface temperature was set at 250°C. In the early development steps, an ionization voltage of 70 eV and a mass range (*m/z*) of 33−200 for total ion chromatogram tracing was used. In the later phases, single ion monitoring was preferred with specific ions of TMA (*m/z*: 42, 44, 58, and 59). Volatile compounds were identified using the NIST10 spectral database, and a comparison of linear retention indices was performed with those of the corresponding pure chemicals.

Electronic nose instrumentation conditions

Electronic nose analysis was performed using a HERACLES II system (Alpha M.O.S., Toulouse, France) and Agilent GC Sampler G6500 CTC PAL Gas Autosampler (Agilent Technologies). The sample was transferred to the headspace vial, heated, and shaken at 250 rpm for 10 min at 50°C for headspace equilibration. A syringe (Hamilton, Reno, NV, USA) was used to inject 2,500 µL of the samples into the headspace. MXT-5 (10 m × 0.18 mm × 0.40 µm) and MXT-1701 (10 m × 0.18 mm × 0.40 µm) (Restek, Bellefonte, PA, USA) columns were used. The oven temperature was initially increased from 40 to 270°C at a rate of 4°C/s. The flame ionization detector temperature was set at 270°C. Volatile compounds were identified using the dedicated AlphaSoft V12 software (Alpha M.O.S., Toulouse, France), and the retention time (RT) and index of the chromatograms obtained using the two columns (MXT 5 and MXT 1701) were matched using the AroChemBase V4 library (Alpha M.O.S.) with modules for chemical measurements and sensory analysis of the properties of the detected compounds.

Statistical processing

SPME-GC/MS results are presented as mean and standard deviation. For statistical analysis between the experimental groups, paired t-tests were performed using R (version 3.1.2). Results were considered significant when the P value was less than or equal to 0.05.

Results and Discussion

TMA content analysis in egg yolk

TMA was analyzed in egg yolk samples by SPME-GC using PDMS/DVB, a mixed type of non-polar and polar SPME fiber to increase versatility, and a DB-WAX column, to selectively analyze polar compounds. Moreover, TMA was qualitatively confirmed by MS, for which the retention time (RT) and characteristic pattern ions were selected to increase the sensitivity of analysis and allow detection at even low concentrations. Analysis of TMA in a water solution confirmed the limit of detection (LOD) to be 0.001 mg/kg. However, due to the matrix effect of the egg yolk, TMA was undetected at concentrations below 0.01 mg/kg, with a signal-to-noise ratio of 3-times the LOD (Table 1). The calibration curve (water samples spiked with TMA) showed excellent linearity ($r^2 \geq 0.99$) in concentration values of 0.05–6 mg/kg. When the calibration curve was validated (egg yolk control samples, $n = 10$), it was confirmed that the linearity was consistently in the range of 0.996–0.999. Since the calibration curve was drawn using the egg yolk matrix, no separate recovery rate validation was performed. Repeatability, expressed as coefficient of variation (CV, %), was determined by analyzing the same samples at least three times on the same day (intra-day precision; CV: 2.31%, $n = 10$) and on consecutive days (inter-day precision; CV: 7.98%, $n = 10$).

Table 1. Trimethylamine validation parameters for the SPME-GC/MS method

Parameter	Analytical component: Trimethylamine
Linearity Range (mg/kg)	0.05–6
r^2 ($n = 10$)	0.996–0.999
Sensitivity	
LOD[a] (mg/kg)	0.01
LOQ[b] (mg/kg)	0.04
Precision	
Intra-day (CV[c] %, $n = 10$)	2.31
Inter-day (CV %, $n = 10$)	7.98

[a] Limit of detection.
[b] Limit of quantification.
[c] Coefficient of variance.

Verification of TMA content in egg yolk after riboflavin-supplemented diet

TMA was detected at a level of 1.06 ± 0.12 mg/kg in hens fed a diet supplemented with choline chloride for 60 days (control sample). A marginal TMA decrease to 0.75 ± 0.07 mg/kg was noted 1 week after the riboflavin-supplemented diet was initiated (Table 2), while it remained stable at 0.25 mg/kg during the following 2–3 weeks, approaching the limit of quantification at week 4. TMA levels were undetected (below the LOD) at week 5. (P value < 0.05). Overall, the TMA content reduced when the riboflavin content was added from the conventional choline chloride-containing diet.

Table 2. Trimethylamine content in egg yolk after riboflavin-supplemented feeding.

Samples	Control (mg/kg)	1 week (mg/kg)	2 weeks (mg/kg)	3 weeks (mg/kg)	4 weeks (mg/kg)	5 weeks (mg/kg)
sample 1	0.87	0.84	0.25	0.17	0.05	ND[a]
sample 2	1.25	0.85	0.26	0.22	0.05	ND
sample 3	1.00	0.82	0.26	0.15	0.05	ND
sample 4	1.22	0.81	0.27	0.34	0.01	ND
sample 5	1.16	0.71	0.27	0.25	0.01	ND
sample 6	0.86	0.82	0.25	0.24	0.03	ND
sample 7	1.20	0.80	0.28	0.31	0.02	ND
sample 8	0.89	0.72	0.25	0.19	0.01	ND
sample 9	0.90	0.84	0.24	0.15	0.09	ND
sample 10	1.23	0.77	0.24	0.29	0.05	ND
sample 11	1.06	0.70	0.26	0.18	0.02	ND
sample 12	1.13	0.68	0.23	0.25	0.01	ND
sample 13	1.02	0.69	0.22	0.28	0.03	ND
sample 14	1.19	0.71	0.28	0.21	0.01	ND
sample 15	1.23	0.78	0.28	0.22	0.05	ND
sample 16	0.87	0.67	0.25	0.29	0.04	ND
sample 17	0.96	0.70	0.22	0.33	0.04	ND
sample 18	0.97	0.82	0.26	0.34	0.02	ND
sample 19	1.19	0.69	0.28	0.31	0.03	ND
sample 20	1.23	0.84	0.27	0.32	0.05	ND
sample 21	1.05	0.65	0.28	0.34	0.05	ND
sample 22	1.08	0.82	0.24	0.28	0.01	ND
sample 23	0.95	0.75	0.22	0.28	0.03	ND
sample 24	1.20	0.88	0.22	0.34	0.13	ND
sample 25	1.07	0.86	0.28	0.24	0.10	ND
sample 26	1.00	0.82	0.28	0.29	0.05	ND
sample 27	1.03	0.69	0.28	0.27	0.05	ND
sample 28	0.89	0.73	0.22	0.26	0.10	ND
sample 29	1.12	0.72	0.25	0.24	0.13	ND
sample 30	1.20	0.68	0.24	0.29	0.05	ND
sample 31	1.12	0.67	0.26	0.23	0.03	ND
sample 32	1.15	0.82	0.26	0.35	0.12	ND
sample 33	1.06	0.85	0.27	0.16	0.05	ND

Table 2. (Continued) Trimethylamine content in egg yolk after riboflavin-supplemented feeding.

Samples	Control (mg/kg)	1 week (mg/kg)	2 weeks (mg/kg)	3 weeks (mg/kg)	4 weeks (mg/kg)	5 weeks (mg/kg)
sample 34	1.08	0.66	0.22	0.22	0.10	ND
sample 35	0.91	0.73	0.28	0.23	0.13	ND
sample 36	0.96	0.76	0.28	0.16	0.10	ND
sample 37	1.15	0.78	0.22	0.33	0.09	ND
sample 38	1.16	0.75	0.26	0.17	0.09	ND
sample 39	1.15	0.72	0.28	0.26	0.05	ND
sample 40	1.04	0.70	0.26	0.26	0.05	ND
sample 41	0.91	0.70	0.24	0.28	0.01	ND
sample 42	1.19	0.72	0.27	0.15	0.01	ND
sample 43	0.93	0.65	0.28	0.20	0.09	ND
sample 44	0.87	0.85	0.28	0.22	0.11	ND
sample 45	0.93	0.87	0.24	0.34	0.11	ND
sample 46	1.07	0.77	0.24	0.17	0.13	ND
sample 47	0.98	0.64	0.25	0.29	0.03	ND
sample 48	0.98	0.68	0.27	0.30	0.10	ND
sample 49	1.12	0.67	0.25	0.16	0.04	ND
sample 50	1.02	0.69	0.26	0.21	0.09	ND
sample 51	1.01	0.69	0.23	0.15	0.01	ND
sample 52	1.21	0.70	0.26	0.20	0.09	ND
sample 53	1.07	0.75	0.24	0.28	0.13	ND
sample 54	0.89	0.88	0.22	0.28	0.02	ND
sample 55	1.16	0.70	0.22	0.35	0.05	ND
sample 56	1.14	0.83	0.28	0.15	0.12	ND
sample 57	0.92	0.74	0.27	0.30	0.03	ND
sample 58	1.23	0.70	0.26	0.30	0.08	ND
sample 59	0.89	0.86	0.24	0.23	0.04	ND
sample 60	1.17	0.74	0.27	0.35	0.05	ND
mean value	1.06	0.75	0.25	0.25	0.06	-
standard deviation	0.12	0.07	0.02	0.06	0.04	-
RSDr(%)[b]	11.37	9.30	8.13	24.83	66.09	-

[a] ND: Not detected or low intensity under limit of detection.
[b] RSDr(%) = standard deviation/mean value * 100.

Analysis of egg yolk volatile compound contents before and after riboflavin supplementation

Next, the volatile compounds present in egg yolk were analyzed by electronic nose, and before and after riboflavin supplementation samples were compared. The volatile compounds detected are listed in Table 3. TMA was detected in the control sample at a RT of 10.89 s using both MXT 5 and 1701 columns. However, no peak corresponding to the RT of TMA was obtained in the egg yolk sample of hens fed for 5 weeks a riboflavin-supplemented diet.

Principal component analysis (PCA), soft independent modeling of class analogies (SIMCA) classification, and statistical quality control (SQC) data were modeled based on the obtained electronic nose data. PCA is the most widely used measurement method for multidimensional data modeling, compression, and visualization, and performed as previously described[19,20]. Based on the

Table 3. Compounds detected by electronic nose in control and 5-week riboflavin-supplemented eggs

Compounds			Control (n = 5)				5-week (n = 5)			
CAS	Formula	Name	RT[1] MXT 5	RI[2] MXT 5	RT MXT 1701	RI MXT 1701	RT MXT 5	RI MXT 5	RT MXT 1701	RI MXT 1701
75-50-3	C_3H_9N	Trimethylamine (TMA)	10.89	417	10.89	436	-	-	-	-
107-02-8	C_3H_4O	Propenal	12.03	450	14.90	560	11.99	449	14.84	558
75-18-3	C_2H_6S	Dimethyl sulfide	13.00	479	14.90	560	12.95	477	14.84	558
75-15-0	CS_2	Carbon disulfide	15.59	554	20.04	672	15.57	553	19.96	670
78-93-3	C_4H_8O	Butan-2-one	16.66	585	20.64	683	-	-	-	-
534-22-5	C_5H_6O	2-Methylfuran	17.20	601	18.09	635	17.16	600	18.02	634
64-19-7	$C_2H_4O_2$	Acetic acid	18.05	615	26.14	773	18.05	615	26.39	776
71-55-6	$C_2H_3Cl_3$	Trichloroethane	18.94	630	20.64	683	-	-	-	-
110-02-1	C_4H_4S	Thiophene	-	-	-	-	20.60	659	23.96	738
2548-87-0	C_8H1_4O	(E)-oct-2-enal	21.09	667	51.57	1,196	-	-	-	-
96-17-3	$C_5H_{10}O$	2-Methylbutanal	-	-	-	-	21.04	666	23.16	725
6032-29-7	$C_5H_{12}O$	Pentan-2-ol	-	-	-	-	23.02	700	28.34	807
554-14-3	C_5H_6S	2-Methylthiophene	27.22	765	28.83	815	27.18	764	28.78	814
4798-44-1	$C_6H_{12}O$	1-Hexen-3-ol	27.89	775	33.97	894	-	-	-	-
13389-42-9	C_8H_{16}	(E)-2-Octene	30.38	813	28.83	815	30.37	813	28.78	814
28588-74-1	C_5H_6OS	3-Furanthiol, 2-methyl-	34.07	871	36.48	934	34.08	871	36.46	934
111-27-3	$C_6H_{14}O$	1-Hexanol	34.59	879	38.47	966	34.58	879	38.46	966
67633-97-0	$C_5H_{10}OS$	2-Pentanone, 3-mercapto-	36.20	904	38.47	966	36.20	904	38.46	966
62-53-3	C_6H_7N	Aniline	40.72	979	48.29	1,136	40.75	980	48.27	1,136
3391-86-4	$C_8H_{16}O$	1-Octen-3-ol	41.72	996	46.13	1,096	41.50	992	46.21	1,098
34300-94-2	$C_5H_{12}OS$	3-Methyl-3-sulfanylbutanol-1-ol	43.60	1,029	47.14	1,115	43.63	1,029	48.27	1,136
13327-56-5	$C_6H_{12}O_2S$	Ethyl 3-(methylthio)propanoate	47.52	1,097	52.09	1,206	-	-	-	-
31823-43-5	$C_9H_{16}O$	3-Nonenal	47.98	1,106	51.57	1,196	-	-	-	-
13679-70-4	C_6H_6OS	2-Formyl-5-methylthiophene	-	-	-	-	47.98	1,106	55.90	1,281
5258-11-07	$C_{10}H_{18}O$	(2R,4R)-tetrahydro-4-methyl-2-(2-methylprop-1-enyl)-2H-pyran	49.57	1,135	51.57	1,196	-	-	-	-
24683-00-9	$C_9H_{14}N_2O$	2-Isobutyl-3-methoxypyrazine	52.80	1,195	52.92	1,223	-	-	-	-
112-40-3	$C_{12}H_{26}$	Dodecane	-	-	-	-	52.85	1,196	52.15	1,208
113486-29-6	$C_{10}H_{18}O_2$	Methylnonanedione	56.28	1,261	61.53	1,395	-	-	-	-
112-12-9	$C_{11}H_{22}O$	Undecan-2-one	58.00	1,294	61.53	1,395	-	-	-	-
629-50-5	$C_{13}H_{28}$	Tridecane	58.75	1,309	56.70	1,296	-	-	-	-
41446-63-3	$C_{14}H_{28}$	E-tetradec-7-ene	63.08	1,392	61.53	1,395	62.95	1,390	62.30	1,412
629-59-4	$C_{14}H_{30}$	Tetradecane	64.23	1,414	61.53	1,395	64.05	1,411	61.44	1,393
3853-83-6	$C_{15}H_{24}$	Alpha-himachalene	66.03	1,448	64.88	1,467	-	-	-	-
629-62-9	$C_{15}H_{32}$	Pentadecane	68.21	1,489	66.21	1,495	-	-	-	-
544-76-3	$C_{16}H_{34}$	Hexadecane	74.24	1,598	71.76	1,615	73.85	1,591	71.63	1,612

[1] Retention Time.
[2] Retention Index.

distance between the yolk samples of hens fed a diet supplemented with riboflavin and those from hens fed a standard diet (Fig. 1a), it can be concluded that the two sample groups have different volatile compounds, as

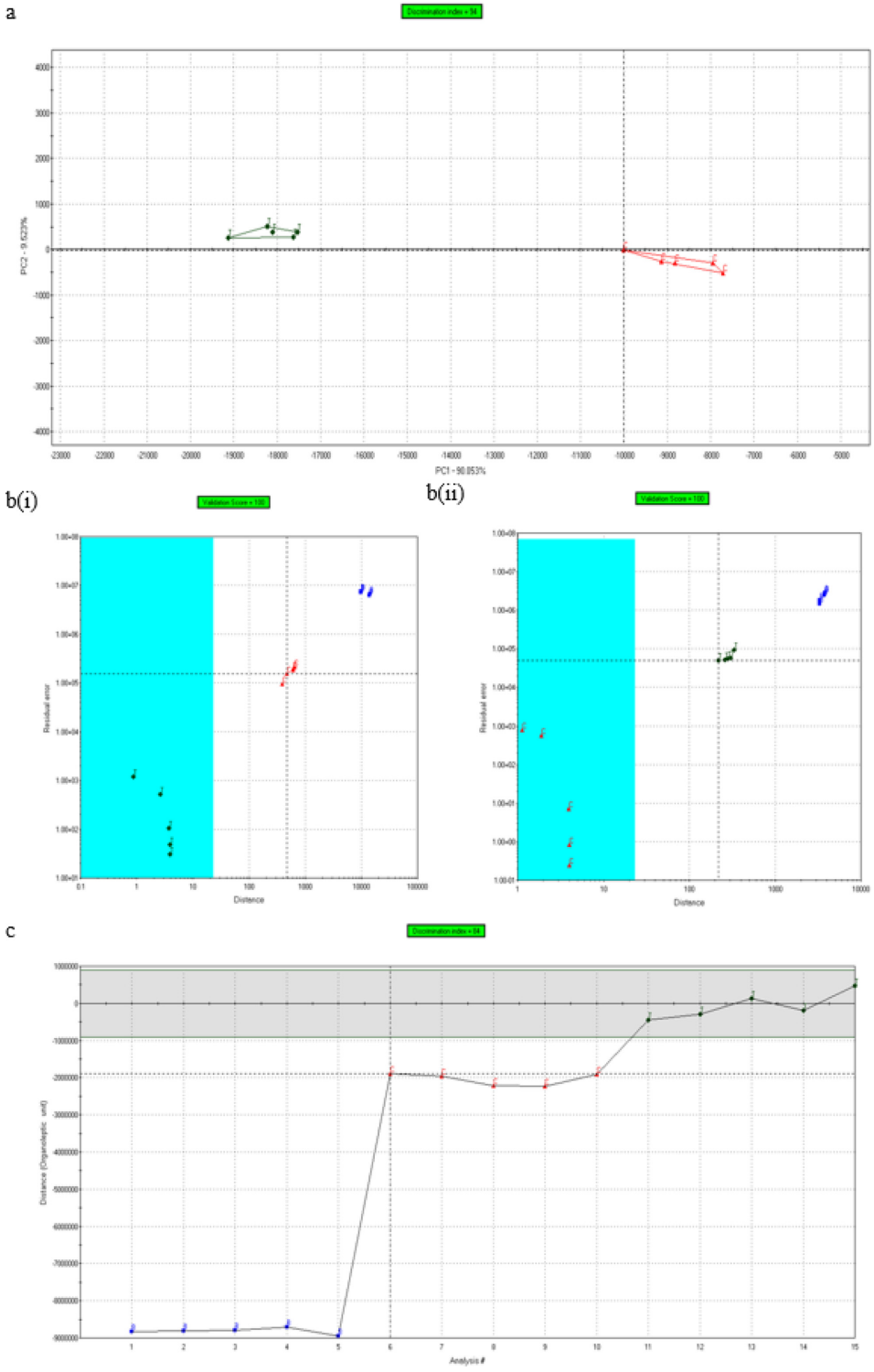

Fig. 1. Electronic nose-based data modeling. a: Principal component analysis. Green and red dots represent yolk samples from hens fed for 6 weeks a diet supplemented with riboflavin and from those fed a standard feed, respectively. b: Soft independent modeling of class analogies classification of data collected from yolk samples of hens fed (i) riboflavin-supplemented diet for 6 weeks (red dots) or (ii) standard feed (green dots). The confidence envelope (certain volume) is shown in light blue. **c:** Statistical quality control data.

shown by the compiled list derived from AroChemBase.

Next, we performed SIMCA modeling, which creates a separate model for each class based on PCA modeling, which is commonly used for sample classification[21, 22].

Significant variation between the blank sample and the remaining sample groups (egg yolk sample or control sample of hens fed riboflavin for six weeks) were observed (Fig. 1b), allowing clear discrimination of the samples.

SQC modeling was used to assess the quality of the analysis process; samples belonging to one group must be in the area specified using the parameter on the Y-axis of the graph[23]. As shown in Fig. 1c, the data within each group showed a small tolerance range, confirming reliability of the results and analysis method. Thus, it is evident that the differences in the Y-axis between the sample groups are significant.

Conclusions

Restriction of choline intake and use of antibiotics are effective in reducing fish odor, but we tried to solve the problems in the direction of maintaining the quality of eggs. Among previous studies, it has been suggested that riboflavin supplementation exhibits the effect of FMO3 enzyme activity[16,17]. It was tested whether hens were fed a supplemental diet of riboflavin to activate the FMO3 enzyme and reduce the content of TMA. Taken together, our findings demonstrate that TMA concentration decreased in eggs laid by hens fed a riboflavin-supplemented diet.

Thus, this approach may induce the conversion of TMA in to TMAO via riboflavin-induced FMO3 activation, and thus reducing the fishy odor of eggs. To further verify this hypothesis, additional analysis of FMO3 activity in hens based while considering different amounts of choline, TMA, TMAO, and riboflavin should be performed. We are planning further investigations to identify and supplement the universal trend by analyzing TMA in egg yolk of commercial eggs.

As mentioned above, further validation of the action of FMO3 is required. However, the TMA reduction effect through riboflavin supplementation has the added advantage of fortifying egg nutrition in addition to the advantages of limiting choline intake and not using antibiotics.

Despite of the limitations of this study, the present findings can contribute to improve the quality and reduce the fishy smell of eggs, thereby enhancing the acceptability of poultry products.

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국문요약

계란의 비린내 강도는 가금류의 종류와 개인의 인식에 따라 다르지만 여러 요인에 의해 발생할 수 있다. 특히 트리메틸아민(TMA)이 원인으로 밝혀졌다. 주목할만한 점은 리보플라빈이 TMA를 무취의 트리메틸아민-N-옥사이드로 전환시키는 역할을 하는 효소인 플라빈 함유 모노옥시게나제 3의 활성을 증가시킬 수 있다는 점이다. 본 연구는 난황의 TMA 함량을 분석하여 비린내에 대한 기여도를 평가하고, 비린내를 방지하는 방법을 개발하는 것을 목적으로 하였다. 고체상 미세추출-기체 크로마토그래피/질량 분석법을 사용하여 리보플라빈이 강화, 보충된 사료를 먹인 암탉의 난황에 있는 휘발성 화합물을 감지하고 정량화했다. 또한 샘플 간 휘발성 물질의 상대적 함량을 비교하기 위해 전자코를 이용하여 상관관계 연구를 수행하였다. TMA는 콜린이 함유된사료를 섭취한 가금류의 난황에서 고농도로 검출되었지만 리보플라빈이 보충된 사료를 섭취한 가금류에서는 검출되지 않았다. 전반적으로, 이 연구는 리보플라빈이 TMA를 포함하여 계란에 존재하는 휘발성 물질의 양과 품질에 영향을 미친다는 것을 시사한다. 이러한 발견이 계란의 비린내를 줄이는 것은 물론 품질 향상에 기여하기를 기대한다.

Conflict of interests

The authors declare no potential conflict of interest.

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