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Effect of Flavin containing monooxygenase (FMO3) genotype on trimethylamine (TMA) content in the chicken egg yolk

Einfluss des Genotypes der Flavinhaltigen Monooxygenase (FMO3) auf den Trimethylamin (TMA)- Gehalt im Dotter des Hühnereies

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Introduction

Important criteria influencing the quality of chicken eggs are taste and smell. An undesirable "fish" taint in eggs reported in the literature is due to the abnormal presence of a tertiary amine, trimethylamine (TMA), which has a smell reminiscent of rotting fish (Humbert et al., 1970; Hobson-Frohock et al., 1973; Griffith et al., 1979; Butler and Fenwick, 1984). TMA is a breakdown product from natural precursors such as choline in the diet. In healthy animals TMA can be absorbed from the intestinal tract and converted to its odourless oxide by the flavin containing monooxygenase (FMO3), a microsomal enzyme in the liver. The oxide is excreted through the gut (Butler and Fenwick, 1984).

It has been shown that the TMA content in chicken egg yolk is influenced by genetic and environmental factors (Butler and Fenwick, 1984; Scholtyssek, 1987). The fish tainted egg problem has been identified in Rhode Island Red and Brown Leghorn chickens but has never been reported in White Leghorn or New Hampshire breeds (Butler and Fenwick, 1984; Horiguchi et al., 1998). Genetically caused fish taint also occurs in mammals. In humans, mutations in the gene coding of the liver enzyme FMO3 lead to a disease called "trimethylaminuria" or "fish odour syndrome" (Dolphin et al., 1997). Individuals suffering from this disease produce a fish taint in sweat, breath and urine. A Swedish Red cattle population has been identified which exhibits this problem in milk due to a single point mutation in the FMO3 gene (Lunden et al., 2002).

Honkatukia et al., (2005) found that a non-synonymous A/T polymorphism in exon 7 of the chicken FMO3 gene leads to an amino acid substitution (T329S) within a highly conserved motif supposedly involved in substrate recognition. Hens homozygous for the polymorphism (TT genotype) showed elevated levels of TMA in egg yolk and are therefore probably not able to metabolize TMA to its odourless oxide.

The aim of the present study was to detect interaction between genotype in chickens resulting from A/T polymorphism in exon 7 of FMO3 gene and deposition of TMA in egg yolk after dietary exposure of Choline, a TMA precursor. To achieve this, a F_2 population was obtained from a cross of White Leghorn cocks (non tainters) and Rhode Island Red hens (tainters). Hens were repeatedly exposed to high dietary levels of choline as a potent TMA precursor to facilitate the identification of differences between hens of different FMO3 genotype.

In order to measure reliably TMA content we had to optimize the colorimetric method for TMA measurement in egg yolk. Several methods for quantifying the concentration of TMA in water, fish muscle, blood and urine have been proposed many years ago (Sivadijan, 1931; Beatty and Gibbson, 1938; Richter, 1938). The earliest colorimetric protocol was described by Richter, (1938) and this was modified by Dyer, (1945) for analysis of the TMA content in fish muscle. Hoogland (1956) and Murray and Gibson, (1972a, b) further developed this procedure and it is presently a routine colorimetric method for determination of TMA, measured as TMA-N in fish muscle. Horiguchi et al., (1998) as well as Jeroch et al., (1999) used the same basic method for analysis of TMA content in the chicken egg yolk. However, in these recent applications in chickens, no standardized protocol was reported.

Material and methods

Animals

In the F_0 generation, 21 Rhode Island Red hens identified as tainters by subjective organoleptic evaluation were cross bred with four cocks of a White Leghorn line. In the latter case, fish taint problems have never been detected. Nine F_1 males were mated to 45 F_1 hens to produce a group of 450 F_2 pullets. Chicks were reared according to standard conditions for laying hens. At 18 weeks of age, the F_2 hens were moved into single cages. Performance traits were recorded including body weight, egg weight, egg shell strength and laying performance.

Out of the 450 F_2 hens, 84 tainters and 84 non tainting half or full sibs were selected based on organoleptic examination. These hens were used to analyse egg yolk TMA content in relation to FMO3 genotype over three feeding periods.

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Feeding

Experimental feeding started when hens aged 43 weeks. The composition of the diet is shown in Table 1. Experimental feeding lasted 18 weeks, and was divided equally into three feeding periods. In the first and third feeding periods, hens were given free access to a diet containing 6000 mg choline/kg while in the second feeding period the diet did not contain any choline supplementation. Three, two and two eggs were collected from each hen in the first, second and third feeding period, respectively.

Chemical Analysis

TMA was extracted from individual egg yolk with 10% trichloroacetic acid (TCA) at a proportion of 15 ml TCA per 15 g egg yolk in an Erlenmeyer flask. After shaking by hand until the mixture was homogeneous, the solution was left at room temperature for at least ten hours. The extract was then filtrated through a folded filter paper (Schleicher and Schuell, Grade $595^1/_2$, for medium-fine precipitates).

For colorimetric analysis, 2 ml of the TCA extract were transferred to a 10 ml glass tube. The extract was made alkaline by the addition of 1.5 ml of a 50% potassium hydroxide solution to separate the amine from its salts. At this point, 0.5 ml of a 10% formaldehyde solution was added to fix other nitrogen containing components. Five ml of toluene was added to extract the TMA. The sample was mixed on a mechanical shaker for two hours at room temperature. Afterwards, 2.5 ml of a 0.02% picric acid solution, diluted in toluene, was added to 2.5 ml of the supernatant to form the characteristic yellow coloured picrate complex (TMA-N picrate). It was subsequently measured photometrically at its maximum absorption wavelength of 410 nm (MURRAY and GIBSON, 1972a). Since only the nitrogen part of TMA forms complexes with picric acid, the results are presented as TMA-N (23.7% of the TMA molecule is nitrogen).

Prior to determination of TMA-N content in egg yolk, a standard curve was established. A dilution series of 16 concentrations was made from a stock solution containing 24 µg TMA-N/ml in deionized water resulting in concentrations ranging from 1 µg to 24 µg TMA-N/ml. Extinction values for ten independent measurements of each of the 16 dilutions were used to establish a TMA-N calibration curve. A total of 30 repeated measurements were made at three different concentrations of six, 12 and 18µg TMA-N/ml to estimate the variation due to the method itself.

Since other nitrogen containing compounds are present in egg yolk, a test was performed to determine the TMA-N concentrations against a background of pooled egg yolk from 30 non-tainted eggs. A graded series was prepared by adding 2, 6, 13, 17 and 24 µg of TMA-N per ml to the egg yolk preparation. Each sample was measured ten times using the established protocol.

To verify measurement results, TMA-N content in egg yolks of 11 hens was analysed by photometric method and gas chromatography applying a modified method of OETJEN and KARL, (1999) using cylcohexane/amyl alcohol (1:1, v:v) instead of tert-butyl methyl ether for extraction. Gas chromatography measurements were done at Federal Research Centre for Fisheries in Hamburg. The used method is based on extraction of TMA by 6% perchloroacid (COMMISSION OF THE EUROPEAN COMMUNITIES, 1995).

Genotyping

All hens were genotyped for a A/T polymorphism at position nt 1034 of the chicken FMO3 cDNA sequence (accession number: AJ4313901), a region in exon 7 of the gene. Individual genotypes AA, AT and TT at this position were determined using DNA isolated from whole blood. Genotyping was done using minisequencing reaction (SnuPe Genotyping, Amersham Biosciences) at Animal Genomics, MTT Agrifood Research Finland as described by HONKATUKIA et al., (2005).

Statistics

As statistical model a two-factorial analysis of variance (ANOVA) was used:

$$y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + \varepsilon_{ijk} \qquad (1)$$

with y_{ijk} = parameter of observation; μ = mean value; a_i = genotype; b_j = feeding period; $(ab)_{ij}$ = interaction and ε_{ijk} = error term. Differences in TMA-N content were tested by multiple comparison using non-parametric Tukeys procedure (JMP, Version 5, SAS Institute Inc). Statistical analysis was performed using software package JMP (Version 5, SAS Institute Inc.).

Table 1. Composition of experimental diet
Zusammensetzung der Futtermischungen

Ingredients (%)	Feeding period I/III	Feeding period II
Wheat	29.8	
Maize	25.0	
Rapeseed extraction meal	10.0	
Soybean, toasted	7.4	
Soybean extraction meal, toasted	7.0	
Soybean extraction meal, HP, toasted	5.0	
Premix	1.5	
Vegetable oil	3.5	
Carbonic acid animal lime	9.0	
Dicalcium phosphate	1.1	
DL-methionine	0.2	
Acid premix	0.4	
Choline (mg/kg)	6000	0

Results

Calibration of the TMA measuring method

Comparing TMA-N standard concentrations against the extinction measurements revealed only marginal differences in curve approximation when values were fitted either to the linear ($R^2 = 0.991$) or quadratic ($R^2 = 0.992$) regression model (Figure 1). As the quadratic approach did not result in a significant improvement in the proportion of variance explained we used the linear function to relate extinction values to absolute TMA-N concentration.

The variation of the measurement protocol was tested by performing thirty independent measurements of each of three different TMA-N concentrations (6, 12 and 18 µg TMA-N/ml). Table 2 shows the results of this test which demonstrated a variation of only 4%.

The specificity of the TCA extraction method is shown in Table 3. Five different amounts of TMA were added to the egg yolk prior photometric measurement. The background variation ranged from 1–6% of the measured value. Recovery rate for these measurements was about 100%.

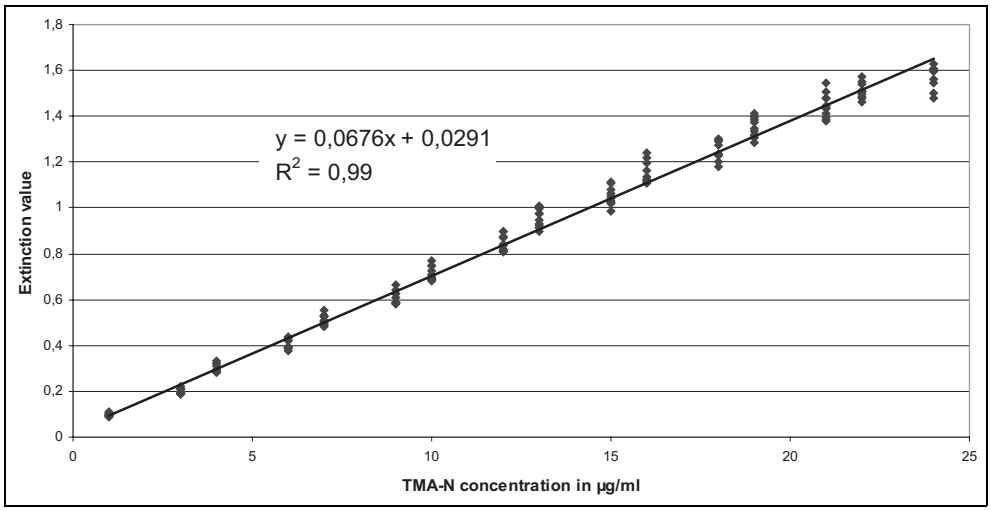

Figure 1. Calibration curves of 16 dilutions of an aqueous TMA-N stock solution containing 24µg TMA-N/ml. *Kalibrierkurven von 16 Verdünnungen einer TMA-N Stammlösung mit 24µg TMA-N/ml.*

Table 2. Extinction values of repeated measurements at three different TMA-N concentrations
Extinktionswerte wiederholter Messungen bei drei verschiedenen TMA-N Konzentrationen

TMA-N [µg/ml]	n	Extinction	SD*	CV** [%]
6.0	32	0.407	0.014	3.4
12.0	31	0.842	0.036	4.3
18.0	32	1.251	0.052	4.2

SD* = Standard Deviation
CV** = Coefficient of variation

Table 3. Repeated measurements of five different TMA-N concentrations in egg yolk extracts
Wiederholte Messungen fünf verschiedener TMA-N Konzentrationen in Eigelbextrakten

Known TMA-N concentration [µg/ml]	Measured TMA-N concentration [µg/ml]; (n=10)	Recovery rate [%]
2.0	2 ± 0	100
6.0	6 ± 0	100
13.0	13.8 ± 0.4	106
17.0	17.2 ± 0.8	102
24.0	23.0 ± 0.7	96

Three eggs collected during the first feeding period were used to calculate the repeatability of TMA-N content of a single hen within a period of one week (data not shown). The repeatability (r) of these individual TMA-N measurements was 0.99 ($p < 0.001$). Therefore, measurements of eggs collected from a hen within one week were combined into a single value in each of the three feeding periods.

Results obtained by the photometric method were confirmed by gas chromatography (Figure 2). In six of the 11 egg yolks, no TMA-N content could be measured by gas chromatography as it was below verification limit of 3 µg TMA-N/g egg yolk. Nevertheless these values were confirmed by the corresponding TMA-N measurements of the photometric method, which equally showed values below 3 µg/g egg yolk. TMA-N contents of the other samples were above verification limit of gas chromatography and showed analogous values to values determined by photometrical analysis.

Analysis of TMA-N content in egg yolk of F_2 hens

TMA-N content in the egg yolk of F2 hens analysed during the three feeding periods is shown in Figure 3.

During the first feeding period with high choline supplementation, no eggs with TMA-N values between 2.1 µg and 5.6 µg TMA-N/g egg yolk were found. Accordingly, hens with TMA-N above 5.6 µg/egg yolk were assigned to the "High Group" while the remaining hens were labelled as "Low Group". The mean TMA-N content of the Low Group was 1.2 ± 0.3 µg/g egg yolk. Thirty of the 84 hens originally identified as Tainters during organoleptical examination had a TMA-N content below 2.1 TMA-N/g egg and clustered in the Low Group. Organoleptical re-testing of the eggs of these 30 hens confirmed misclassifying them as Tainters in the first organoleptic examination. The High Group with 54 animals had a mean value of 13.2 ± 3.6 µg TMA-N/g egg yolk ranging from 5.6 to 23.4 µg/g.

Resumption of the choline rich diet in the third period demonstrated a significant difference between hens of High (13.0 ± 4.4 µg TMA-N/g) and Low Group (0.8 ± 0.2 µg TMA-N/g). All animals which had appeared in the High Group during the first exposure to a choline rich diet displayed again high TMA-N concentrations in egg yolk. All hens of the Low Group had definable low TMA-N content. The Spearman Rank correlation coefficient between TMA-N content in egg yolk of hens in the High Group during the two periods of choline supplemented diet was at $r = 0.62$ ($p < 0.001$).

In the second feeding period (without choline supplementation), the TMA-N contents in egg yolk from all hens were low (mean 0.9 ± 0.2 µg TMA-N/g egg yolk), and there was no significant difference between High and Low Group. The TMA-N content in the egg yolk measured in the feeding period without supplemented choline can be regarded as basic TMA-N level. The diet contained rapeseed meal and therewith sinapin as a possible precursor for TMA. TMA-N content in egg yolk was not correlated with other performance traits including body weight, egg weight, shell strength or monthly laying performance (data not shown). None of these parameters showed significant differences between High and Low Group.

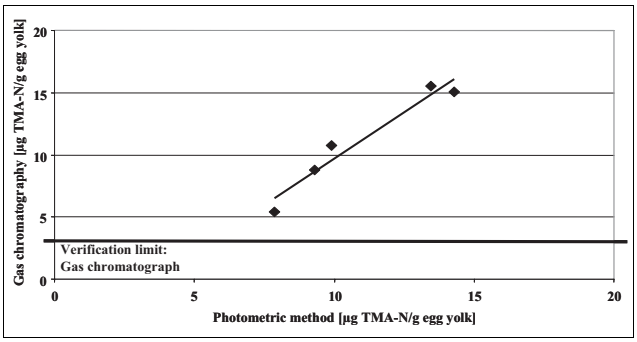

Figure 2. Representation of the relation between TMA-N contents measured by gas chromatography and by photometric method.
Darstellung der Beziehung zwischen TMA-N Konzentrationen, gaschromatographisch und photometrisch gemessen.

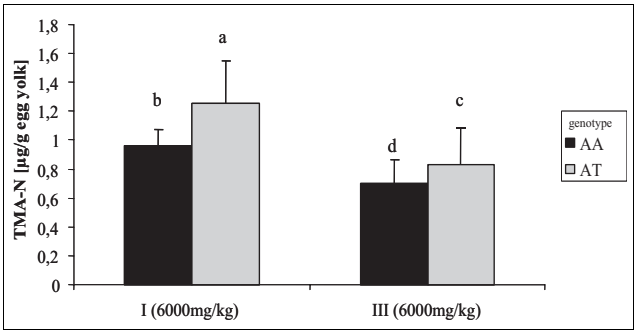

Figure 4. Differences of TMA-N content in egg yolks between the FMO3 genotypes AA and AT in two high-choline supplemented feeding periods (different letters mean significantly different TMA-N levels, $p < 0.05$).
Unterschiede im TMA-N Gehalt von Eigelb zwischen den FMO3 Genotypen AA und AT in zwei mit hoher Cholinmenge supplementierten Fütterungsperioden (unterschiedliche Buchstaben bedeuten signifikant verschiedene TMA-N Level, $p < 0.05$).

Association of FMO3 Genotype with TMA-N content

Genotyping of the hens revealed that all hens of homozygous TT genotype exhibited high levels of TMA-N content in egg yolk and formed exclusively the High Group, while hens of AA and AT genotype belonged to the Low Group. Moreover, significant differences were found in TMA-N content between hens of AA and AT genotypes (Figure 4). Eggs from heterozygous hens contained significantly more TMA-N than eggs of hens of genotype AA in the first and third feeding period ($p < 0.01$). Furthermore, hens of genotype AA as well as hens of genotype AT exhibited higher amounts of TMA-N in the first feeding period than in the third one. The TMA-N content of TT hens was much higher and did not differ between the first and the third feeding period while lower values were found in the third feeding period in both AA and AT hens.

Discussion

Olfactory deviations in chicken eggs lead to complaints by consumers. It is known that fish smell in egg yolk originates from TMA. The current study was performed to compare deposition of TMA in egg yolks after feeding TMA precursors between F2 hens which differ in their FMO3

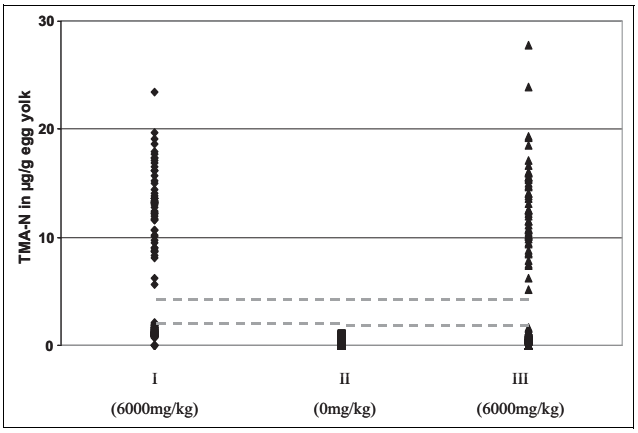

Figure 3. Distribution of TMA-N content in egg yolks of F2 hens for feeding period I (6000mg/kg), II (0mg/kg) and III (6000 mg/kg). The area without measurement is shown by two broken lines.
Verteilung des TMA-N Gehalts im Eidotter von F2 Hennen in den Fütterungsphasen I (6000mg/kg), II (0mg/kg) und III (6000 mg/kg). Der Bereich ohne Messwerte ist durch zwei gestrichelte Linien gekennzeichnet.

genotypes resulting a non-synonymous A/T polymorphism in exon 7. This polymorphism is correlated with fishy taint of chicken eggs. To investigate the differences in deposition of TMA in the egg yolk between defined FMO3 genotypes, it was first necessary to establish a standardised method to analyse the TMA-N content in egg yolks. We used a photometric method based on publications by Murray and Gibson, (1972a) for TMA-N measurements in fish muscle. Testing the repeatability of the established method we found that the mean coefficient of variation of 60 repeated measurements of each of three standard dilutions of TMA-N was low with only 4%. This supports the observation by Murray and Gibson, (1972a, b) that the photometric method is highly reliable and stable.

Our analyses used isolated egg yolk. Previous studies in laying hens based on whole eggs and reported lower values of TMA than those found in the present study (Pearson et al., 1983). This is due to the fact that 95% of unoxidised TMA is localised in the egg yolk (Hobson-Frohock et al., 1973). The purification success was confirmed by examining the recovery rate of known amounts of TMA-N added to egg yolk of unaffected hens. Using the linear calibration curve established in this study a recovery rate of about 100% for TMA-N of these standards were obtained. This is consistent with earlier reports (Murray and Gibson, 1972a; Shen, 1988) based on TCA/formalin purification. In addition, results obtained by the photometric method were confirmed by gas chromatography (Figure 2). Taken together, the method promises to be a useful and objective tool for analysis of TMA-N as fish taint causing agent in chicken eggs. It can be used for testing genetic differences as in the present study.

In our investigation, we used a dietary challenge with choline as a model to detect individuals suffering from reduced capability to eliminate TMA. Pearson et al., (1983) observed, that feeding hens with high amounts fish meal-rich diet as source of TMA precursors leads only in certain animals to fish tainted eggs. Other hens fed with the same diet remained unaffected. Another precursor for TMA is sinapin (Butler and Fenwick, 1984), which is present in rapeseed. As in the current study the used diets in all three feeding periods equally contained 10% of rapeseed extraction meal (Table 1), TMA converted from

sinapin did not play any significant role in differences of the TMA contents between groups in the different feeding periods. In our experiment, the first and third feeding period with choline enriched diet clearly divided hens into High and Low Group according to TMA-N content in egg yolks. Although the two-peaked distribution of TMA-N might be partly attributed to the preselection made in the total set of F_2 hens, the 30 hens misclassified by organoleptical evaluation as tainters all displayed TMA-N measurements below 2.1 µg/g egg yolk (threshold of the Low Group). This might rather support a two-peaked distribution in the population. Misclassification also showed that organoleptical examination of eggs is a quite subjective method and not sufficiently confident for classifying hens into Tainters and Non-Tainters.

None of the recorded performance traits were significantly different between High and Low group indicating that there is no association between TMA-N content of egg yolks and body weight, laying performance, egg weight or shell strength. This is in agreement with findings by PEARSON et al., (1983) who did not find significant differences in body weight between two organoleptically classified groups of hens.

After interrupting the choline challenge in the second feeding period the TMA-N content in the egg yolks of all hens decreased, and no significant differences between the groups were detected. PEARSON et al., (1983) reported, that the TMA content in eggs dropped down when a fish meal-rich diet was withdrawn. Interestingly, they identified a small group of hens which continued to produce eggs with high TMA content even after cessation of the fish meal rich diet. A similar observation was reported by ZENTEK and KAMPHUES, (2000). Hens laid tainted eggs (identified by organoleptical examination) despite the fact that no specific precursors of TMA were fed. The cause of these different results to our findings that TMA-N decreases in all F_2 hens when no supplemental choline is fed, is not clear. However, there is a possibility that the effect is dependent on differences in the genetic make up of the chickens used in the different experiments. It is also possible that other genetic factors than the A/T polymorphism in exon 7 of the chicken FMO3 gene are involved in metabolism of TMA and may modulate the capacity to oxidise TMA. In humans it is known that the strength of fish taint is dependent on which of several mutations of the FMO3 gene is involved (KRUEGER, 2002). In some cases, individuals can control the fish taint through diets which do not contain precursors of TMA (ZSCHOCKE and MAYATEPEK, 2000) but in other cases, individuals suffer from fish taint even without consumption of TMA precursors (TREACY et al., 1998; AKERMAN et al., 1999; FORREST et al., 2001).

The results obtained by comparing TMA-N content in egg yolk between FMO3 genotypes showed that hens being homozygous TT for the polymorphism in exon 7 of the FMO3 gene lost their ability to oxidize TMA and exhibited high amounts of TMA in their eggs, which make them smell of rotting fish. Although hens genotyped for AA and AT both were able to metabolise choline to the odourless TMA-oxide, differences in the concentrations of TMA-N in the egg yolks were found. This might suggest that the A to T polymorphism is inherited as semi-dominant trait and animals of the AT genotype have a limited, but enhanced ability to metabolize TMA compared to animals of AA genotype.

The animals of the TT genotype did not show any differences in TMA-N content in the first and third feeding period (high choline supplementation). However, within both AA and AT genotypes, differences in TMA-N content in the egg yolk in dependence on the feeding period (age of the hen) were found. The eggs of both genotypes contained significantly more TMA-N in the first feeding period than in the third one. DÄNICKE et al., (2006) found significantly lower TMA concentrations in 72 week old hens than in 47 week old hens. Other studies showed that TMA content increases with age (JEROCH et al., 1995) and duration of feeding contaminated feed (PEARSON et al., 1983). It seems that an age effect might exist on the TMA metabolising ability in laying hens.

As it is not sufficiently known about differential behaviour of AA and AT genotype on the deposition of TMA in egg yolks in relation to feed compounds and environmental or individual factors, further investigations are required.

Acknowledgement

We thank Mrs. Annett Weigend for technical help to establish photometric analysis of TMA-N in egg yolk.

Summary

Choline is a precursor of TMA which causes a smell reminiscent of rotting fish preferentially in brown shelled eggs of chickens. Previously it has been shown that a non-synonymous A/T polymorphism in exon 7 of the chicken Flavincontaining Monooxygenase gene (FMO3) leads to an amino acid substitution (T329S) within a highly conserved motif which is supposedly involved in substrate recognition. The present study aimed to determine the effect of genotype resulting from this A/T polymorphism on the TMA content in egg yolks after feeding a choline rich diet. To determine phenotypic differences, first a photometric method was established to analyse the TMA content in egg yolks. In the experiment, 21 Rhode Island Red hens which were specifically selected for laying smelling eggs ("Tainters") were crossed with four White Leghorn males assumed to be "Non-Tainters" to produce F_2 hens. These hens were genotyped and TMA content in eggs was determined. In the first and third feeding period the diet was supplemented with 6000 mg choline/kg. Hens with TMA-N content greater than 5.6µg/g egg yolk were assigned to "High Group". This group showed exclusively TT genotype. In contrast, hens with a TMA-N content below 2.1 µg/g egg yolk were assigned to "Low Group" and had both AA and AT genotypes. Within the Low Group, eggs of AT hens showed significantly higher TMA-N levels in the egg yolks than AA hens did. Identical distribution was found in third feeding period. In contrast, no differences were observed between groups when feeding of the choline rich diet was discontinued in the second feeding period.

Key words

Chicken egg taint, trimethylamine, choline, FMO3 genoype

Zusammenfassung

Einfluss des Genotypes der Flavinhaltigen Monooxygenase (FMO3) auf den Trimethylamin (TMA)-Gehalt im Dotter des Hühnereies

Cholin ist eine Vorstufe für die Bildung von TMA, das vorzugsweise bei braunschaligen Hühnereiern einen an

fauligen Fisch erinnernden Geruch verursacht. Es wurde gezeigt, dass beim Huhn ein nicht-synonymer A/T Polymorphismus im Exon 7 des Gens der Flavinhaltigen Monooxigenase 3 (FMO3) zu einem Aminosäureaustausch (T329S) in einer hochkonservierten Region führt, die vermutlich an der Substraterkennung beteiligt ist. Die vorliegende Studie hat zum Ziel, den Einfluss des Genotypes, der aus dem A/T Polymorphismus resultiert, auf den TMA-Gehalt im Eidotter nach Fütterung einer cholinreichen Diät zu untersuchen. Um phänotypische Unterschiede feststellen zu können, wurde eine photometrische Methode zur Analyse des TMA-Gehalts im Eidotter etabliert. Zur Erstellung von F2 Hennen für die Untersuchungen wurden 21 Rhode Island Red Hennen, die organoleptisch als Stink-Eier legende Tiere („Tainter") identifiziert worden waren, mit vier White Leghorn Hähnen verpaart, die als „Nicht-Tainter" klassifiziert wurden. Für diese Hennen wurde der FMO3 Genotyp und der TMA-N Gehalt im Eidotter bestimmt. In der ersten und dritten Fütterungsphase war das Futter mit 6000 mg Cholin/kg Futter angereichert. Hennen, die einen TMA-N Gehalt größer als 5,6 µg/g Eidotter aufwiesen, bildeten die „High Group". Die Hennen dieser Gruppe waren ausschließlich vom TT Genotyp. Im Gegensatz dazu bildeten die Hennen mit weniger als 2,1 µg TMA-N/g Eidotter die „Low Group" und hatten entweder AA- oder AT-Genotyp. Innerhalb der „Low Group" wiesen die Eier der AT Hennen signifikant höhere TMA-N Gehalte auf als die Eier der AA Hennen. Eine identische Verteilung der Hennen wurde in der dritten Fütterungsphase gefunden. Im Gegensatz dazu konnten während der zweiten Fütterungsphase, in der das Futter nicht mit Cholin angereichert war, keine Unterschiede zwischen den Gruppen beobachtet werden.

Stichworte

Fischgeruch, Hühnerei, Timethylamin, Cholin, FMO3 Genotyp

References

AKERMAN, B.R., H. LEMASS, L.M. CHOW, D.M. LAMBERT, C. GREENBERG, C. BIBEAU, O.A. MAMER and E.P. TREACY, 1999: Trimethylaminuria is caused by mutations of the FMO3 gene in a North American cohort. Molecular Genetic Metabolism **68** (1), 24-31.

BEATTY, S.A. and N.E. GIBBONS, 1937: The measurement of spoilage in fish. Journal of the Biological Board of Canada **3**, 77-91.

BUTLER, E.J. and G.R. FENWICK, 1984: Trimethylamine and fish taint in eggs. World's Poultry Science Journal **40**, 38-51.

COMMISSION OF THE EUROPEAN COMMUNITIES, 95/149/EC, 1995: Commission Decision of 8 March 1995 fixing the TVB-N (total volatile basic nitrogen) limit values for certain categories of fishery products and specifying the analysis methods to be used. *Official Journal* **L097**, 29/04/1995, p. 84-87.

DÄNICKE, S., K.H. UEBERSCHÄR, K. REESE and S. WEIGEND, 2006: Investigations on the effects of rape oil quality, choline and methionine concentrations in diets for laying hens on the Trimethylamine (TMA) content of the eggs, on TMA metabolism and on laying performance. Archive of Animal Nutrition **60** (1), 57-79.

DOLPHIN, C.T., J.H. RILEY, R.L. SMITH, E.A. SHEPARD and I.R. PHILLIPS, 1997: Structural organisation of the human flavin-containing monooxygenase 3 gene FMO3, the favoured candidate for fish odor syndrome, determined directly from genomic DNA. Genomics **46** (2), 260-267.

DYER, W.J., 1945: Colorimetric Determination of Trimethylamine as the picrate salt. Journal of the Fisheries Research Board of Canada **6**, 351-358.

FORREST, S.M., M. KNIGHT, B.R. AKERMAN, J.R. CASHMAN and E.P. TREACY, 2001: A novel deletion in the flavin-containing monooxygenase gene (FMO3) in a Greek patient with trimethylaminuria. Pharmacogenetics **11**, 169-174.

GRIFFITH, N.M., D.G. LAND and A. HOBSON-FROHOCK, 1979: Trimethylamine and egg taint. British Poultry Science **20** (6), 555-558.

HOBSON-FROHOCK, A., D.G. LAND, N.M. GRIFFITH and R.F. CURTIS, 1973: Egg taints. association with trimethylamine. Nature **243** (5405), 304-305.

HONKATUKIA, M., K. REESE, R. PREISINGER, M. TUISKULA-HAAVISTO, S. WEIGEND, J. ROITO, A. MÄKI-TANILA and J. VILKKI, 2005: Fishy taint in chicken eggs is associated with a substitution within a conserved motif of the FMO3 gene. Genomics **86 (2)**, 225-232.

HOOGLAND, P.L., 1958: Grading fish for Quality 2. Statistical Analysis of the Result of Experiments Regarding Grades and Trimethylamine Values. Journal of the Fisheries Research Board of Canada **15** (4), 717-728.

HORIGUCHI, K., K. SHIMIZU, K. TOTSUKA, A. YAMAMOTA, T. ITOH, S. FUIJIMURA and T. ISHIBASHI, 1998: White Leghorn hens supplied excess choline, rapeseed meal or fish meal produce fish odor free eggs. Journal of Animal Science and Technology (Jpn.) **69,** 22-25.

HUMBERT, J.A., K.B. HAMMOND, W.E. HATHAWAY, J.G. MARCOUX and D. O'BRIEN, 1970: Trimethylaminuria, The fish odor Syndrome. Lancet i, 770-771.

JMP® STATISTICS AND GRAPHIC GUIDE: Version 5; © Copyright 1995 by SAS Institute Inc., Cary, NC, USA.

JEROCH, H., S. DÄNICKE and R. ZACHMANN, 1995: Zum Futterwert und zur Eignung von Rapsexpellern in der Legehennenfütterung. Agribiol. Res. **48** (3-4), 248-256.

JEROCH, H., S. DÄNICKE, J.G. BRETTSCHNEIDER and W. SCHUHMANN, 1999: Einsatz von behandelter Rapssaat bei braunen Legehennen. Austrian Journal of Agricultural Research **1,** 45-54.

KRUEGER, S.K., D.E. WILLIAMS, M.F. YUEH, S.R. MARTIN, R.N. HINES, J.L. RAUCY, C.T. DOLPHI, E.A. SHEPARD and I.R. PHILLIPS, 2002: Genetic polymorphisms of flavin-containing monooxygenase (FMO). Drug metabolism reviews **34** (3), 523-532.

LUNDEN, A., S. MARKLUND, V. GUSTAFFSON and L. ANDERSSON, 2002: A nonsense mutation in the FMO3 gene underlies fish off-flavour in cow's milk. Genome Research **12**, 1885-1888.

MURRAY, C.K. and D.M. GIBSON 1972a: An investigation of the method of determining trimethylamine in fish muscle extracts by the formation of its picrate salt. Journal of Food Technology **7**, 35-47.

MURRAY, C.K. and D.M. GIBSON 1972b: An investigation of the method of determining trimethylamine in fish muscle extracts by the formation of its picrate salt. Journal of Food Technology **7**, 47-51.

OETJEN, K. and H. KARL, 1999: Improvement of gas chromatographic determination methods of volatile amines in fish and fishery products. Deutsche Lebensmittelrundschau **95**, 403-407.

PEARSON, A.W., N.M. GREENWOOD, E.J. BUTLER, C.L. CURL and G.R. FENWICK, 1983: Fishmeal and egg taint. Journal of the Science of Food and Agriculture **34**, 277-285.

RICHTER, D., 1938: Elimination of amines in man. Biochemical Journal **32** (19), 1763-1769.

SCHOLTYSSEK, S., 1987: Geflügelprodukte. Verlag Eugen Ulmer. p 31-135.

Shen, M., 1988: Spectrophotometric Determination of Trimethylamine Nitrogen in Raw Waters using picric acid. The Analyst **113**, 1139-1140.

Sivadijan, J., 1931: J. Pharm. chim. **13**, 529.

Treacy, E.P., B.R. Akerman, L.M. Chow, R. Youil, C. Bibeau, J. Lin, A.G. Bruce, M. Knight, D.M. Danks, J.R. Cashman and S.M. Forrest, 1998: Mutations of the flavin-containing monooxygenase gene (FMO3) cause trimethylaminuria, a defect in detoxication. Human Molecular Genetics **7** (5), 839-845.

Zentek, J. and J. Kamphues, 2000: Geruchsabweichungen bei Eiern brauner Hennen – Vorkommen auch bei „unkritischer" Futterzusammensetzung möglich. Deutsche Tierärztliche Wochenschau **107**, 355-358.

Zschocke, J. and E. Mayatepek, 2000: Biochemical and molecular studies in mild flavin monooxygenase 3 deficiency. Journal of Inherited Metabolic Disease **23**, 378-382.

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