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Interactions of flavin containing monooxygenase 3 (FMO3) genotype and feeding of field beans and rapeseed cake on the trimethylamine (TMA) content in egg yolks of laying hens

Interaktionen zwischen Flavinhaltiger Monooxigenase 3 (FMO3) und der Fütterung von Ackerbohnen und Rapskuchen auf den Trimethylamin (TMA)-gehalt im Eidotter von Legehennen

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Introduction

In the past the occurrence of fishy tainted eggs was a well known problem in brown egg laying hens. The taint is caused by the tertiary amine trimethylamine (TMA) (Hobson-Frohock et al., 1973). TMA occurs naturally in fish meal or is produced by fermentation by enteric bacteria from precursors such as choline and sinapine (March and MacMillan, 1979). In healthy chickens, TMA can be converted to its odourless oxide by the microsomal liver enzyme flavin containing monooxygenase 3 (FMO3) and excreted through the gut (Butler and Fenwick, 1984, Bain et al., 2005). Honkatukia et al. (2005) found a single base change (A to T) at position nt1034 of the coding sequence in exon 7 of FMO3 gene (gi: 18873598) in chicken which leads to an amino acid change (T329S) within a highly conserved motif involved in substrate recognition that impairs the ability of the hens organism to oxidize TMA. Thus, fishy-egg tainting can be described as nutrigenetic condition because both genetic disposition and dietary TMA precursors must be present for egg tainting to appear. Due to modern breeding programs laying hens homozygous for the SNP are not longer present in commercial flocks. Nevertheless commercial and scientific interest exists on relations between FMO3 genotypes, especially the heterozygous AT genotype, feeding and the TMA content in chicken eggs. The inhibitory effect of glucosinolates such as goitrin and other antithyroid compounds which are commonly contained in rapeseed products is well documented (Fenwick et al., 1981, Pearson et al., 1981, Goh et al., 1983). Several methods are established to reduce the content of these compounds in feed stuffs including selection of cultivars of rapeseed with low glucosinolate content (Hickling, 2001) and treatment procedures (Jeroch et al., 1997). Other field crops in the focus for animal feeding are grain legumes as field beans. They are considered as good protein and starch source (Abel et al., 2004). But field beans contain the antinutritive substances tannins, vicine, convicine and lectins. The content of these antinutritive substances limits the use in animal diets. Due to these compounds field beans negatively affect growth, performance, histological structures and proteolytic activities in liver and other organs of chicken (Santidrián et al., 1987; Ortiz et al., 1994; Halle, 2005). Because of the ability to change histological structures and activities of liver enzymes, vicine and convicine may be able to inhibit the FMO3 mediated oxidation of TMA. It is not yet investigated if these antinutritive compounds act as inhibitor on the enzyme FMO3 and therewith influence the occurrence of fishy tainted eggs, even in chicken without genetic disposition.

The aim of the present study was to evaluate the influence of potential FMO3 inhibitors in combination with TMA precursors in the diet on the TMA-N content in egg yolks of laying hens being homozygous or heterozygous at nucleotide position 1034 of the coding sequence in exon 7 of FMO3 gene. To achieve this goal, two different experiments were conducted. In experiment 1, hens of different genetic background were used. Diets were supplemented with high amounts of the TMA precursor choline. Furthermore, untreated and hydrothermally treated field beans, respectively, as feed ingredient containing potential FMO3 inhibitors were included in the diets. In experiment 2 the effect of feeding an increasing amount of rapeseed cake, which contains naturally the TMA precursor sinapine and glucosinolates as FMO3 inhibitor, on TMA-N content in egg yolks was studied.

Material and methods

Experimental Design

To investigate the influence of a field bean containing diet on the TMA-N content in chicken eggs in relation to the FMO3 genotype, a feeding experiment with Rhode Island Red pure breed hens and Lohmann Brown commercial layers was conducted. The experimental setup is shown in Figure 1. Experiment 1 was divided into two periods. In period 1 the animals were fed either a control diet or diets containing 4000 mg choline/kg, 4000 mg choline/kg and 100 g of untreated or hydrothermally treated field beans/kg diet. The experimental diets were fed for twenty weeks. At the end of period 1 five animal per dietary group were

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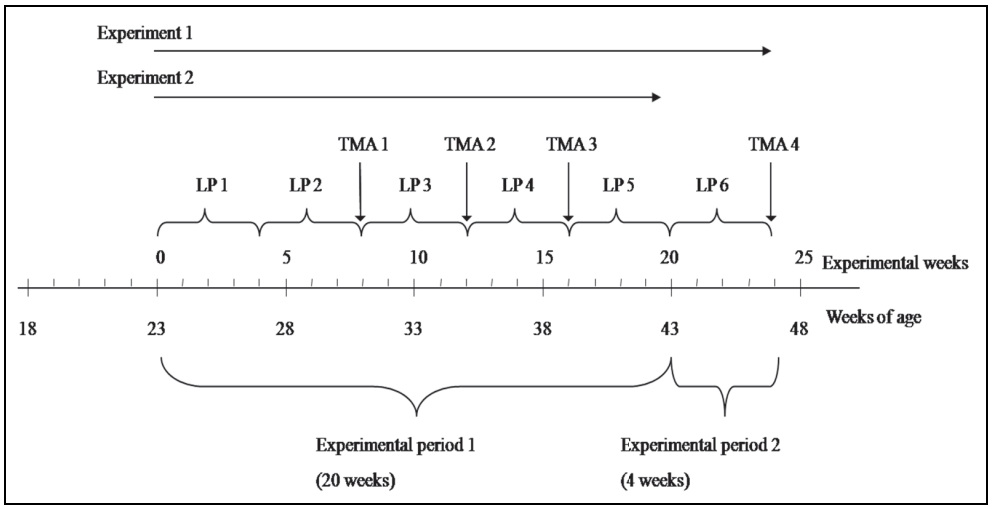

Figure 1. Experimental design. TMA 1-4 = point in time for collection of eggs for TMA analysis in egg yolks, LP 1-6 = Laying period (à 28 days)
Experimentelles Design. TMA1-4 = Zeitpunkt der Sammlung der Eier für die TMA-Analyse in den Eidottern, LP 1-6 = Legeperiode (à 28 Tage)

slaughtered for tissue analyses which are not subject of the present work. In period 2 the respective amounts of untreated and hydrothermally treated field beans were elevated from 100 g/kg to 200 g/kg diet while the other two diets remained unchanged. This period lasted four weeks.

In experiment 2 the influence of two concentrations of rapeseed cake in the diet on TMA-N content in egg yolk was tested. Hens of the Lohmann Brown commercial layer line were fed a control diet or diets containing 100 and 300 g rapeseed cake/kg, respectively, for twenty weeks.

Animals of both experiments were housed in single cages in a two-floor battery. Feed intake and laying performance were recorded individually. Hens were fed a commercial layer diet until 23 weeks of age. Within experimental periods, subdivided into 28-day periods of laying, the laid eggs were recorded daily. A total of eight eggs were collected from each hen to measure egg weight within each laying period. Feed was offered *ad libitum* in meal form. Residual feed was weighted every week. Water was supplied by nipple drinkers. As performance parameters for each 28-day laying period were calculated: the number of laid eggs, egg weight, egg mass, feed-to-egg mass ratio, daily feed intake and laying intensity.

Animals

All chickens used in this study were genotyped at position nt1034 of the cDNA sequence (AJ431390), a region in exon 7 of the chicken FMO3 gene that is associated with variation in the TMA content in the egg yolk of laying hens. To obtain the animals for the experiment, cocks and hens of a Rhode Island Red (RIR) pure bred line from commercial breeding program (Lohmann Tierzucht GmbH, Cuxhaven, Germany) were genotyped at this polymorphic site of the genome. Only heterozygous chickens were used as parents to achieve all three FMO3 genotypes (TT, AT, AA) in the next generation. Hens of the FMO3 genotype TT were used as positive TMA control and therewith show that there are enough TMA precursors in the experimental diets to provoke the FMO3 system. Genotypes were assigned to the experimental diets as shown in Table 1. In total, 216 of the RIR daughters were used for the experiment. In addition, Lohmann Brown (LB) laying hybrids, which resulted from a cross of RIR with another pure line, were genotyped for the A/T polymorphism to identify AA and AT types. Two hundred and sixteen (144 for feeding with field beans, 72 for feeding with rapeseed cake) LB hens were used in the experiment.

DNA-isolation and genotyping

After isolation of DNA from whole blood by a phenol-chloroform method, the FMO3 genotypes were analysed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique as described previously (Kretzschmar et al., 2009).

Diets

Dietary composition. According to the methods of the Verband Deutscher Landwirtschaftlicher Untersuchungsanstalten (Vdlufa, Naumann and Basler, 1993) the diets were analysed for nitrogen and dry matter. An overview over components, calculated and analysed composition of the experimental diets is given in Table 2. Vicine and convicine contents in field beans and diets were analysed by a HPLC method (Quemener, 1988) by RD-Biotech Expertises, Services et Kits en Analyses Biologiques, Besançon, France. Contents of extractable condensed tannins in field beans and diets were analysed as leucocyanidinequivalents by butanol-HCL extraction (Jayanegara et al., 2011). Contents of analysed antinutritive substances in feed stuffs and diets are summarised in Table 3.

Field bean treatment. Investigations in experiment 1 were carried out with the field bean variety Limbo which is a coloured blooming, tannin rich species. The field beans were subjected to a hydrothermal treatment to reduce tannine, vicine and convicine contents. The process includes an opening with sodium bicarbonate, long-time conditioning, expansion and drying of the treated material. The hydrothermal treatment was conducted by Amandus Kahl Nachf., Reinbek, Germany and is described in detail by Jeroch et al. (1997). Not treated and hydrothermally treated field beans were included in the experimental diets to evaluate if the treatment to reduce the secondary compounds has an effect on the formation of TMA-N in the egg yolks.

TMA-N analysis

Eggs for TMA-N analysis were collected in experimental weeks 8, 12, 16 and 24 (Figure 1). The egg yolks from two eggs of each hen were pooled for TMA-N analysis. TMA was extracted from the pooled sample with 10% trichloroacetic acid and analysed as TMA-N by a photometric method as described by Kretzschmar et al. (2007).

Table 1. Distribution of hens according to the FMO3 genotype throughout experiment 1 and 2
Verteilung der Hennen in Abhängigkeit vom FMO3-Genotyp während Experiment 1 und 2

Diet	FMO3 genotype				
	RIR[1]-AA[2]	RIR-AT[2]	RIR-TT[2]	LB[3]-AA	LB-AT
Control (500 mg choline/kg diet)	18	18	18	18	18
(Experiment 1, Period 2)	(10)	(13)	(13)	(13)	(11)
Experiment 1					
Period 1					
4000 mg choline/kg diet	18	18	18	18	18
4000 mg choline + 100 g field beans/kg diet	18	18	18	18	18
4000 mg choline + 100 g t[4] field beans/kg diet	18	18	18	18	18
Period 2					
4000 mg choline/kg diet	13	13	13	13	13
4000 mg choline + 200 g field beans/kg diet	13	13	13	13	13
4000 mg choline + 200 g t[4] field beans/kg diet	13	13	13	13	13
Experiment 2					
100 g rapeseed cake/kg diet				18	18
300 g rapeseed cake/kg diet				18	18

[1] RIR, Rhode Island Red pure breed animals
[2] AA, AT, TT, FMO3 genotype
[3] LB, Lohmann Brown commercials
[4] hydrothermally treated

Statistical analyses

TMA-N content in egg yolks

For statistical analysis of measured TMA-N contents in egg yolks non parametric Kruskal-Wallis test with subsequent Median-test was used.

Performance parameters

The effects of FMO3 genotypes in combination with supplementation of choline and untreated or hydrothermally treated field beans to the diets on performance parameters in experiment 1 followed a 5 by 2 factorial analysis of variance (ANOVA). The variation within individual hens when measured more than once in the course of the experiment was considered as random effect (effect of repeated measurements):

$$y_{ijkl} = \mu + a_i + b_j + c_k + (a \cdot b)_{ij} + (a \cdot c)_{ik} + (b \cdot c)_{jk} + (a \cdot b \cdot c)_{ijk} + d_{l(a \cdot b)} + e_{ijkl}$$

with y_{ijkl} = parameter of observation for genotype i, choline addition j and laying month k; μ = overall mean, a_i = genotype (RIR-AA, RIR-AT, RIR-TT, LB-AA, LB-AT); b_j = experimental diet (500 mg choline/kg, 4000 mg choline/kg, 4000 mg choline + 100 g field beans/kg, 4000 mg choline + 100 g hydrothermally treated (t) field beans/kg); c_k = laying month; $(a \cdot b)_{ij}$, $(a \cdot c)_{ik}$, $(b \cdot c)_{jk}$, $(a \cdot b \cdot c)_{ijk}$ = interactions; $d_{l(a \cdot b)}$ = effect of repeated measurements (consecutive laying months) within the same hen 1, e_{ijkl} = error term.

For experiment 2 a similar statistical model as for experiment 1 was used, but with b_j = rapeseed cake supplementation in the diet (0, 100 and 300 g/kg).

All statistical analyses were performed using STATISTICA 6.1 software from StatSoft® Inc.

Results

Experiment 1. TMA-N contents in egg yolk of different genotypes

TT-hens. TMA-N analysis in the 8th week of experimental feeding showed that RIR-TT hens of all experimental groups, except control group, had significantly higher amounts of TMA-N in their eggs than the hens of all the other feeding groups (Figure 2.1). Among the three experimental diets no significant differences between TMA-N levels in egg yolk of RIR-TT hens were found (median expressed as µg TMA-N/g egg yolk, 4000 mg choline/kg diet = 15.9, 4000 mg choline + 100 g field beans/kg diet = 16.4, 4000 mg choline + 100 g t field beans/kg diet = 13.9). For RIR-TT hens, similar results were found in the 12th and 16th week of experimental feeding (Figures 2.2 and 2.3). For all diets, these animals always exhibited significantly higher amounts of TMA-N in egg yolk than the hens of all other FMO3 genotypes (median expressed as µg TMA-N/g egg yolk, 12th week: 4000 mg choline/kg diet = 16.0, 4000 mg choline + 100 g field beans/kg diet = 19.5, 4000 mg choline + 100 g t field beans/kg diet = 17.9; 16th week: 4000 mg choline/kg diet = 17.3, 4000 mg choline + 100 g field beans/kg diet = 20.0, 4000 mg choline + 100 g t field beans/kg diet = 16.3). Moreover, in the 24th week of experimental feeding (period 2 of experiment 1), when hens of the two field bean containing diet groups were fed for four weeks with 200 g untreated or hydrothermally treated field beans/kg diet, no significant differences in levels of TMA-N in the egg yolks were found (median expressed as µg TMA-N/g egg yolk, 4000 mg choline/kg diet = 15.9, 4000 mg choline + 200 g field beans/kg diet = 18.1, 4000 mg choline + 200 g t field beans/kg diet = 16.3, Figure 2.4).

Table 2. Composition of experimental diets [g/kg]
Zusammensetzung der Versuchsfuttermischungen [g/kg]

	Control (500 mg choline/kg diet)	4000 mg choline/kg diet	4000 mg choline + 100 g field beans/kg diet	4000 mg choline + 100 g t[1] field beans/kg diet	100 g rapeseed cake/kg diet	300 g rapeseed cake/kg diet
Components:						
Soybean meal	216	216	169.3	169.3	144	0
Dicalcium phosphate	23	23	24.2	24.2	21	16
Calcium carbonate	80	80	79.2	79.2	80	80
Sodium chloride	3.2	3.2	4.0	4.0	2.7	2.0
DL-methionine	0.9	0.9	1.1	1.1	0.7	0.4
Soybean oil	26	26	34.1	34.1	16	0
Maize	635.9	635.9	573.1	573.1	620.6	586.6
Field beans	0	0	100	0	0	0
Field beans, treated	0	0	0	100	0	0
Rapeseed cake	0	0	0	0	100	300
Premix 500[2]	15	0	0	0	15	15
Premix 4000*	0	15	15	15	0	0
Calculated composition:						
Crude protein [g/kg]	153	153	153	153	153	153
Crude fat [g/kg]	55	55	61	61	57	66
ME [MJ]	11.79	11.79	11.8	11.8	11.76	11.79
Analysed Composition:						
DM [g/kg]	895	893	894	899	894	896
Crude protein [g/kg DM]	157	160	164	164	167	167

[1] t hydrothermally treated,
[2] provided per kg diet: 40 mg Fe, 16 mg Cu, 80 mg Zn, 100 mg Mn, 0.25 mg Se, 1.2 mg I, 10000 IU vitamin A, 2500 IU vitamin D3, 20 mg vitamin E, 4.0 mg vitamin K3, 2.5 mg vitamin B1, 7 mg vitamin B2, 4 mg vitamin B6, 20 µg vitamin B12, 10 mg pantothenic acid, 40 mg nicotinic acid, 25 µg biotin, 0.6 mg folic acid, 125 mg butylated hydroxytoluene, 1.0 mg beta-apo-8'-carotinal, 4 mg canthaxanthine, 575 mg choline chloride, * Provided similar amounts of vitamins and minerals per kg diet as premix 500 but was completed with 4600 mg choline chloride/kg

Table 3. TMA-relevant substances in feed stuffs and diets
TMA-relevante Substanzen in Futtermitteln und Futtermischungen

	Analysed substance				
Feed stuff/diet	Sinapine [mg/kg]	Glucosinolates [mmol/kg DM]	Tannins [% DM]	Vicine [µg/g]	Convicine [µg/g]
Control (500 mg choline/kg diet)	n.d.[1]	n.d.	n.a.[2]	n.a.	n.a.
4000 mg choline/kg diet	n.a.	n.a.	n.a.	n.a.	n.a.
4000 mg choline + 100 g field beans/kg diet	n.a.	n.a.	0.03	499	331
4000 mg choline + 100 g t[3] field beans/kg diet	n.a.	n.a.	0.02	655	436
100 g rapeseed cake/kg diet	750	1.5	n.a.	n.a.	n.a.
300 g rapeseed cake/kg diet	2600	5.0	n.a.	n.a.	n.a.
Field beans	n.a.	n.a.	1.52	5767	3444
Field beans hydrothermally treated	n.a.	n.a.	1.31	4918	2862
Rapeseed cake	11030	16.1	n.a.	n.a.	n.a.

[1] n.d., not detectable
[2] n.a., not analysed
[3] t, hydrothermally treated

AA and AT hens. TMA-N content in egg yolks did not differ significantly between consecutive measurements within RIR-AA, RIR-AT, LB-AA and LB-AT hens of all three analyses performed during experimental feeding (Figure 2.1: 8th week, Figure 2.2: 12th week, Figure 2.3: 16th week) (median expressed as µg TMA-N/g egg yolk: 8th week, lowest value 0.421 (4000 mg choline + 100 g field beans/kg diet, RIR-AA), highest value 1.32 (4000 mg choline/kg

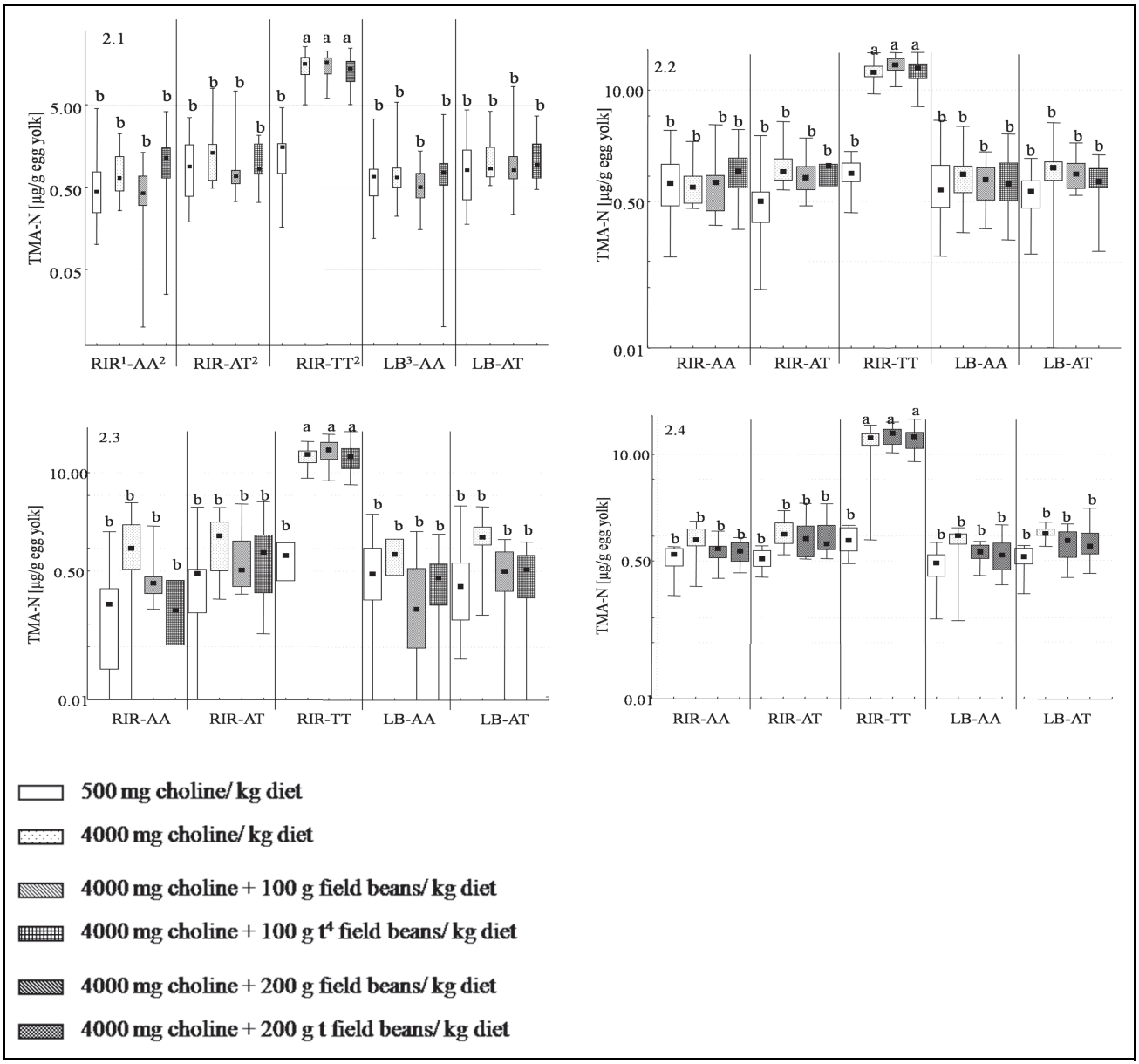

Figure 2. Experiment 1. TMA-N content in egg yolks. Dependence of TMA-N content on field bean (not treated or hydrothermally treated) content in feed and FMO3 genotype of the hens. 2.1. 8th week of experiment. 2.2. 12th week of experiment. 2.3. 16th week of experiment. 2.4. 24th week of experiment. Different superscripts indicate significantly different TMA-N contents ($p < 0.05$). [1] RIR: Rhode Island Red pure breed animals, [2] AA, AT, TT: FMO3 genotype, [3] LB: Lohmann Brown commercials, [4] t: hydrothermally treated, data is logarithmic presented on axis of ordinates

Experiment 1. TMA-N-Gehalt in Eidottern. Abhängigkeit des TMA-N-Gehaltes von Ackerbohnengehalt (nicht behandelt oder hydrothermisch behandelt) im Futter und dem FMO3-Genotyp der Hennen. 2.1. 8th Woche im Experiment. 2.2. 12th Woche im Experiment. 2.3. 16th Woche im Experiment. 2.4. 24th Woche im Experiment. Unterschiedliche Buchstaben bedeuten signifikant verschiedene TMA-N-Gehalte ($p < 0.05$). [1] RIR: Rhodeländer Reinzuchttiere, [2] AA, AT, TT: FMO3-Genotyp, [3] LB: Lohmann Brown Hybriden, [4] t: hydrothermisch behandelt, Daten auf der Ordinatenachse sind logarithmisch dargestellt

diet, RIR-AT), 12th week, lowest value 0.511 (control, RIR-AT), highest value 1.31 (4000 mg choline + 100 g t field beans/kg diet, RIR-AT), 16th week, lowest value 0.182 (control, RIR-AA), highest value 1.46 (4000 mg choline/ kg diet, RIR-AT)).

In addition, comparison of the TMA-N values in the 24th week of the experiment (period 2), after feeding 4 weeks 200 g untreated or hydrothermally treated field beans/kg diet, no significant differences between TMA-N content in egg yolks of the hens of the experimental feeding groups including the control group were measured (median expressed as µg TMA-N/g egg yolk, lowest value 0.470 (control, LB-AA), highest value 1.07 (4000 mg choline/kg diet, LB-AT), Figure 2.4).

Experiment 2. TMA-N contents in egg yolks of different feeding groups

No significant differences in TMA-N contents in the egg yolk from hens of the different experimental groups were found in the 8th week of experimental feeding with increasing concentrations of rapeseed cake (median expressed

as µg TMA-N/g egg yolk, control LB-AA = 0.684, LB-AT = 0.791, 100 g rapeseed cake/kg diet LB-AA = 0.886, LB-AT = 0.687, 300 g rapeseed cake/kg diet LB-AA = 0.457, LB-AT = 0.871). Similar results were found in the 12th and 16th week, respectively, of experimental feeding. Results showed no differences in TMA-N content in the egg yolk of hens, neither between FMO3 genotypes AA and AT nor between the three dietary treatments (data not shown).

Experiment 1. Performance parameters

Results of the laying performance parameters are presented in Table 4. Laying performance was influenced by genetic background. LB-hens produced eggs with about 5% more weight and 7% higher egg mass. These hens had a 7% higher feed intake than RIR-hens. Dietary treatment was not found to influence any of the investigated performance parameters. A significant effect of the period was found, reflecting the characteristic progression of the laying performance throughout the whole laying period. Interactions of genotype and period were significant for feed intake and laying intensity.

The increase of the field bean content in the diets in experimental week 20 did not have any effect on the performance of the animals. In Figure 3 (a) comparison of the egg mass as example of performance parameters in laying period five and six is given. Egg mass was significantly lower for the hens of the dietary group with 4000 mg choline and 100 g (laying period 5)and 200 g (laying period 6) field beans/kg diet, respectively, than for the hens of the other feeding groups (Figure 3(a)). LB-hens of FMO3 genotype AA and AT showed higher egg mass in both laying periods compared with RIR hens (Figure 3(b)).

Table 4. Performance of laying hens in comparison of control group and experiment 1 in dependence on FMO3 genotype and not treated and treated field bean concentration (23–43 weeks of age)
Leistung der Legehennen im Vergleich der Kontrollgruppe und der Fütterungsgruppen aus Experiment 1 in Abhängigkeit von FMO3-Genotyp und unbehandeltem und behandelten Ackerbohnengehalt (23–43 Lebenswoche)

Genotype	Diet	Egg Weight [g]	Feed intake [g/d]	Laying Intensity [%]	Egg mass [g/d]	Feed to egg mass ratio [g/g]
RIR[1]-AA[2]	Control	59.6	111	96.3	57.8	1.95
RIR-AA	4000 mg Choline/kg diet	59.0	116	98.7	58.3	2.00
RIR-AA	4000 mg Choline + 100 g field beans/kg diet	58.9	112	95.8	56.6	2.02
RIR-AA	4000 mg Choline + 100 g t[4] field beans/kg diet	58.4	114	97.1	56.8	2.01
RIR-AT[2]	Control	60.8	117	98.1	59.7	1.98
RIR-AT	4000 mg Choline/kg diet	57.8	104	92.2	54.4	1.98
RIR-AT	4000 mg Choline + 100 g field beans/kg diet	58.1	113	97.7	56.8	2.01
RIR-AT	4000 mg Choline + 100 g t field beans/kg diet	58.5	109	94.9	55.6	1.99
RIR-TT[2]	Control	59.2	112	96.4	57.3	1.98
RIR-TT	4000 mg Choline/kg diet	59.9	109	92.8	57.8	1.96
RIR-TT	4000 mg Choline + 100 g field beans/kg diet	58.9	110	96.4	56.9	1.96
RIR-TT	4000 mg Choline + 100 g t field beans/kg diet	59.6	113	96.7	57.7	1.97
LB[3]-AA	Control	62.9	121	98.2	61.8	1.98
LB-AA	4000 mg Choline/kg diet	62.9	120	97.8	62.0	1.96
LB-AA	4000 mg Choline + 100 g field beans/kg diet	62.1	123	98.5	61.1	2.02
LB-AA	4000 mg Choline + 100 g t field beans/kg diet	60.8	122	98.5	59.9	2.04
LB-AT	Control	62.6	118	98.9	61.9	1.91
LB-AT	4000 mg Choline/kg diet	63.4	122	98.7	62.6	1.97
LB-AT	4000 mg Choline + 100 g field beans/kg diet	62.1	118	96.4	60.0	1.99
LB-AT	4000 mg Choline + 100 g t field beans/kg diet	61.4	116	97.9	60.1	1.95
ANOVA (Probability)						
Genotype		< 0.01	< 0.01	0.02	< 0.01	0.18
Diet		0.09	0.77	0.39	0.06	0.27
Period		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Genotype × diet		0.67	0.05	0.11	0.25	0.92
Genotype × period		0.30	< 0.01	< 0.01	0.38	0.29
Diet × period		0.85	0.32	0.83	0.39	0.01
Genotype × diet × period		0.47	0.61	0.06	0.31	0.70
PSEM		0.192	0.238	0.566	0.007	0.003

[1] RIR: Rhode Island Red pure breed animals
[2] AA, AT, TT: FMO3 genotype
[3] LB: Lohmann Brown commercials
[4] t: hydrothermally treated

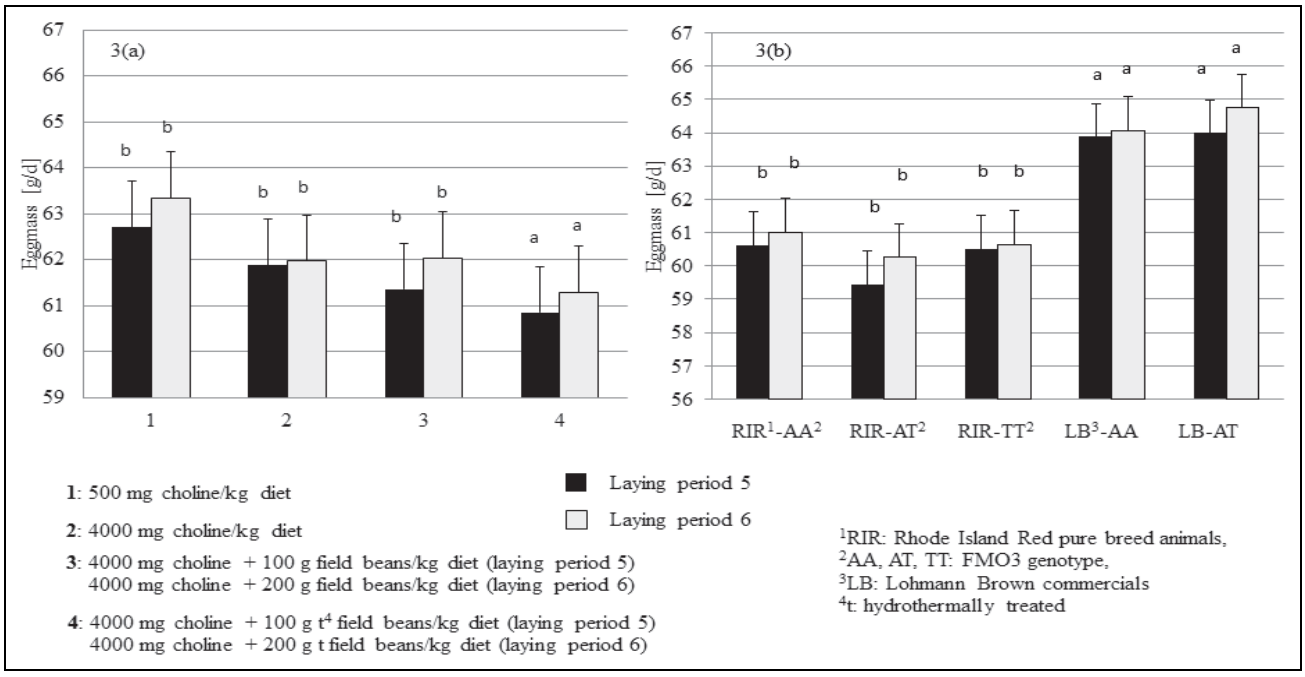

Figure 3. Experiment 1. Egg mass as performance parameter for laying hens in dependence on feeding group (3(a)) and FMO3 genotype (3(b)) in laying periods 5 and 6, respectively. Different superscripts indicate significantly different egg mass ($p < 0.05$). *Experiment 1. Eimassse als Leistungsparamter für Legehennen in Abhängigkeit von der Fütterungsgruppe (3(a)) und dem FMO3-Genotyp (3(b)) in den Legeperioden 5 und 6. Unterschiedliche Buchstaben bedeuten signifikant verschiedene Eimasse ($p < 0.05$).*

Experiment 2. Performance parameters

No differences in performances were found between LB-hens of the FMO3 genotypes AA and AT (Table 5). The addition of rapeseed cake to the diet significantly decreased feed intake, egg weight, egg mass, feed-to-egg-mass ratio and laying intensity. Layers of the 300 g rapeseed cake/kg diet feeding group had eaten about 15% less feed than control group animals and laid eggs with 10% lower weight and 11% lower egg mass. Significant interactions between rapeseed cake addition and period were found, leading to lower egg weight, egg mass and feed-to-egg mass ratio. Decreases in feed intake, egg weight, egg mass and feed-to-egg mass ratio were caused by interactions of diet and period (Table 5).

Discussion and conclusions

The main goal of the study was to examine if components naturally occurring in feed stuffs can act as FMO3 inhibitors and therewith contribute to the occurrence of fishy taint in eggs. In the present experiment, hens of the FMO3 genotypes AA and AT displayed amounts of TMA-N in egg yolks under sensory detection limit of about 1.0 µg TMA-N/g egg yolk (Hobson-Frohock et al., 1973), independent of the experimental feeding group. Only TT-hens displayed higher TMA-N levels in egg yolks when fed the high choline supplemented diets. It has previously been described that enriching a layer's diet with 4000 mg choline provokes slightly but significantly elevated TMA levels in AT hens (Kretzschmar et al., 2009). In the present experiment such enrichment was used to ensure the presence of sufficient TMA precursors to request the TMA oxidation system.

To evaluate the influence of FMO3 inhibitors, field beans were included in the diets. Field beans contain vicine and convicine (Mager et al., 1980) and tannins (Perez-Maldonado et al., 1999). These compounds are known as antinutritive substances. The used field bean variety Limbo is a coloured blooming, tannin rich species. Analysed vicine and convicine concentration in the untreated field beans are comparable to results presented by other investigators (Rahmann et al., 2007, Halle, 2005). The hydrothermal treatment reduced vicine concentration to 85% and convicine concentration to 83% of the original concentration, respectively. Tannin concentration was reduced to 86% by hydrothermal treatment. The incorporation of untreated and hydrothermally treated field beans into the experimental diets of the current experiment did not have any effect of the TMA-N amounts in the egg yolks. Neither AA-hens nor AT-hens of the field bean groups produced higher amounts of TMA-N in their eggs.

Vicine and convicine are found to increase liver proteolytic activity in male chicken, when fed to a diet containing 500 g raw field beans/kg (Santidrián et al., 1984). Larralde (1982) describes changes in the activities of enzymes associated with amino acid catabolism and intestinal digestion caused by feeding raw field beans to rats and chickens. Ortiz et al. (1994) report negative influences on the histological structure of intestine and liver depending on field bean tannins at concentrations of 8 and 16 g/kg diet, respectively. Because of these described influences on liver enzymes and intestine an inhibitory effect of feeding field beans on the liver enzyme FMO3 can be supposed. While in these mentioned experiments (Santidrián et al., 1984; Ortiz et al., 1994) 1-day-old male chicks were used, the results of the current study are based on feeding experiments with laying hens (23 weeks old). Differences in the sensibility of the digestive system depending on age may be responsible for oppositional findings. Furthermore, much higher concentrations of field beans and field bean tannins, respectively, than in the own experiment were used. It can be concluded that the concentration of tannins, vicine and convicine naturally occurring in the used field

Table 5. Performance of laying hens in comparison of control group and experiment 2 in dependence on FMO3 genotype and rapeseed cake concentration (23–43 weeks of age)
Leistung der Legehennen im Vergleich der Kontrollgruppe mit den Fütterungsgruppen aus Experiment 2 in Abhängigkeit vom FMO3-Genotyp und der Rapskuchenkonzentration (23–43 Lebenswoche)

Genotype	Diet	Egg weight [g]	Feed intake [g/d]	Laying intensity [%]	Egg Mass [g/d]	Feed to egg mass ratio [g/g]
LB[1]-AA[2]	Control	62.9	121	98.2	61.8	1.98
LB-AA	100 g rapeseed cake/kg diet	61.7	117	98.8	61.0	1.92
LB-AA	300 g rapeseed cake/kg diet	56.5	103	96.6	55.3	1.89
LB-AT[2]	Control	62.6	118	98.9	61.9	1.91
LB-AT	100 g rapeseed cake/kg diet	61.7	112	99.2	58.7	1.91
LB-AT	300 g rapeseed cake/kg diet	57.1	102	96.8	55.4	1.89
ANOVA (Probability)						
FMO3 Genotype		0.25	0.12	0.63	0.41	0.33
Diet		< 0.01	< 0.01	0.04	< 0.01	0.21
Period		< 0.01	< 0.01	0.03	< 0.01	< 0.01
Genotype × diet		0.14	0.78	0.96	0.39	0.57
Genotype × period		0.32	0.75	0.50	0.43	0.16
Diet × period		< 0.01	< 0.01	0.73	0.02	< 0.01
Genotype × diet × period		1.00	0.70	0.55	0.80	0.83
PSEM		0.323	0.408	0.970	0.014	0.004

[1] LB: Lohmann Brown commercials
[2] AA, AT: FMO3 genotype

beans, which belongs to the tannin rich species, do not affect the FMO3 enzyme system of laying hens. The used chickens were able to produce non-tainting eggs even if the TMA precursors choline and sinapine and the antinutritive substances vicine and convicine were present in the diet.

In the current experiment, a negative impact on laying performance was found with 100 and 200 g hydrothermally treated field beans/kg diet only for egg mass for all hens in the 5th and 6th laying period (Figure 3(a)). All the other investigated performance parameters were not negatively influenced by field bean diets. The negative effects of field bean containing chicken diets on performance parameters are extensively reported. Santidrián et al. (1984) found reduced growing rate in chicken fed with 500 g field beans/kg diet, Perez-Maldonado et al. (1999) describes a decrease in egg mass, egg weight, feed intake and laying intensity caused by 250 g field beans/kg diet. Halle (2005) reports massive loss of hens when 200 g field beans or more were incorporated per kg diet. With 100 g field beans/kg diet reduced feed intake, laying intensity, egg weight and egg mass were documented. On the other hand, Rahmann et al. (2007) could not manifest any influence of 100 g field beans/kg diet on laying performance and animal health. In contrary to the mentioned investigations with comparable amounts of field beans and their antinutritive substances, in the current experiment no worse effect of elevating field bean concentration to 200 g/kg diet could be found (data not shown).

Previous studies indicated that hens of the FMO3 genotypes AA and AT are sufficiently able to metabolise the TMA precursor sinapine from rapeseed and rapeseed products and no fishy taint in eggs occurs (Ward et al., 2009; Kretzschmar et al., 2009). In these studies hens were fed up to 240 g canola meal/kg diet (Ward et al., 2009) or 100 and 300 g rapeseed cake/kg diet, respectively, for four weeks (Kretzschmar et al., 2009). The results of the current experiment confirm these findings. Even after 16 weeks of feeding the animals with 300 g rapeseed cake/kg diet no elevated TMA-N contents in egg yolks of AA- and AT-hens were detected.

Well documented is the negative impact of rapeseed product in chicken's diet on laying and performance parameters (Jeroch et al., 1995, Richter et al., 1996, Jeroch et al., 1997). The results of the present experiment confirm with these findings. 100 g rapeseed cake/kg diet can be given to layers without resulting in reduction of laying performance. More than 100 g/kg diet is not recommended because of downgrading feed intake, egg weight, egg mass and feed-to-egg mass ratio.

As main results of the experiments can be concluded that neither field beans nor rapeseed cake in the used amounts and compositions are able to inhibit or overload the FMO3 enzyme system in laying hens of the FMO3 genotypes AA and AT. Obviously one intact allele of the FMO3 gene is sufficient to synthesize a functioning enzyme which eliminates TMA effectively from blood, and consequently hens do not transfer TMA to eggs. Only the reduction of laying performance by feeding field beans and rapeseed cake should be taken into consideration.

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Summary

The aim of the present study was to evaluate the relationship between Flavincontaining monooxygenase 3 (FMO3) genotype and dietary FMO3 inhibitors on trimethylamine (TMA)-N content in egg yolks of laying hens. Fishy taint is characteristic for the tertiary amine TMA. Rhode Island Red pure breed hens and Lohmann Brown commercial layers were genotyped for non-synonymous A to T polymorphism at position nt1034 of the cDNA of the FMO3 gene. This polymorphism is associated with variation in TMA-N levels in egg yolks. Hens of the FMO3 genotype AA, AT and TT were used for the experiments. Genotypes were equally distributed among the varying experimental diets. As dietary FMO3 inhibitors natural or hydrothermally treated field beans (100 g/kg diet) or rapeseed cake (0, 100 and 300 g/kg diet) were incorporated in the diets. Field beans contain tannins, vicine and convicine as antinutritive substances. Rapeseed cake contains the TMA-precursors glucosinolates and sinapine. TMA was extracted from egg yolks with trichloroacetic acid and analysed as TMA-N by a photometric method. The performance parameters number of laid eggs, egg weight, laying intensity, egg mass, daily feed intake and feed-to-egg-mass ratio were recorded. High amount of TMA-N was found in egg yolk of TT-hens when fed with the experimental diets. In contrast, hens of the AA-genotype and heterozygous AT-hens only exhibited low levels of TMA-N. 300 g rapeseed cake/kg diet reduced performance of hens. No negative effect on performance could be found in the experimental groups fed on natural and hydrothermally treated field beans, respectively. It can be concluded both field beans and rape seed cake at concentrations of antinutritive substances used in this experiment can be fed to brown egg layer hens of the FMO3 genotypes AA and AT without leading to fishy tainted eggs.

Key words

Laying hens, trimethylamine, FMO3 genotype, field beans, rapeseed cake

Zusammenfassung

Interaktionen zwischen Flavinhaltiger Monooxigenase 3 (FMO3) und der Fütterung von Ackerbohnen und Rapskuchen auf den Trimethylamin (TMA)-gehalt im Eidotter von Legehennen

Das Ziel der vorliegende Untersuchung war es, den Zusammenhang zwischen dem Genotyp der flavinhaltigen Monooxigenase 3 (FMO3) und FMO3-Hemmern, die in der Nahrung vorkommen, auf den Trimethylamin (TMA)-N-Gehalt in Eidotter von Legehennen zu untersuchen. Das tertiäre Amin TMA verursacht Fischgeruch in Hühnereiern. Für die Versuche wurden Hennen einer Reinzucht Rhodeländerlinie und Lohmann Brown Hybriden auf den nicht-synonymen A zu T Polymorphismus an Position nt1034 der cDNA des FMO3 Gens typisiert. Dieser Polymorphismus steht in engem Zusammenhang mit Veränderungen im Gehalt an TMA-N in Eidottern. Für die Experimente verwendet wurden Hennen der drei FMO3-Genotypen AA, AT und TT. Die Hennen wurden entsprechend ihres FMO3 Genotyps gleichmäßig auf die experimentellen Gruppen verteilt. Als dietätische FMO3-Hemmer wurden jeweils unbehandelte und hydrothermisch behandelte Ackerbohnen (100 g/kg Futter) sowie Rapskuchen (0, 100 und 300 g/kg Futter) in die Futtermischungen eingearbeitet. Ackerbohnen enthalten als antinutritive Substanzen Tannine, Vicin und Convicin. In Rapskuchen sind Glucosinolate und Sinapin enthalten, die als TMA-Vorstufen fungieren. Das TMA wurde mit Trichloressigsäure aus den Eidottern extrahiert und als TMA-N mittels einer photometrischen Methode bestimmt. Als Leistungsparameter wurden für jede Henne Zahl der gelegten Eier, Eigewicht, Eimasse, Legeintensität, tägliche Futteraufnahme sowie das Verhältnis von Futteraufnahme zu Eimasse bestimmt. Hohe Gehalte an TMA-N wurden ausschließlich bei den Tieren des TT-Genotyps in den experimentellen Fütterungsgruppen gefunden. Im Gegensatz dazu waren bei den Hennen der FMO3-Genotypen AA und AT nur geringe Mengen an TMA-N im Eidotter nachzuweisen. Bei der Fütterung von 300 g Rapskuchen/kg Futter reduzierte sich die Leistung der Tiere signifikant. Keine negativen Effekte auf die Legeleistung konnte bei der Fütterung mit unbehandelten als auch mit hydrothermisch behandelten Ackerbohnen gefunden werden. Es kann geschlussfolgert werden, dass sowohl Ackerbohnen als auch Rapskuchen mit den hier verwendeten Gehalten an antinutritiven Substanzen in Futtermischungen für Braunleger der FMO3-Genotypen AA und AT verwendet werden können, ohne zu Eiern mit nennenswerten TMA-Gehalten und damit Fischgeruch zu führen.

Stichworte

Legehennen, Trimethylamin, FMO3 Genotyp, Ackerbohnen, Rapskuchen

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