TRIMETHYLAMINE PRODUCTION FROM SINAPINE BY ENTERIC BACTERIA FROM LAYING HENS

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Vondell (1948) found that "rotten-egg" odor noted occasionally in eggs was associated with bad breath in layers and Hobson-Frohock et al. (1973) have shown that latter is due to dimethyl sulphide (DMS). In contrast, Hobson-Frohock et al. (1973) showed that the "fishy" odor of eggs sometimes noted in eggs laid by brown-shelled egg layers fed rations containing rapeseed meal (RSM) was due to trimethylamine (TMA). The source of the TMA in such eggs has been reported by Hobson-Frohock et al. (1977) and Clandinin et al. (1977) to be sinapine which is present in rapeseed meal at a level of about 1.5%.

Since sinapine contains choline and since choline has been shown (Dyer and Wood, 1947; DE LA Huerga and Popper, 1952; Popper et al., 1952 and Prentiss et al., 1961) to be converted to TMA by intestinal bacteria it was decided to determine whether inoculum taken from the caeca of chickens is capable of splitting sinapine (as sinapine bisulfate) to yield choline as a substrate for TMA formation and some other compound as a substrate for DMS production.

MATERIALS AND METHODS

Three Rhode Island Red hens which had previously produced "fishy" eggs when fed rations containing RSM and which had been fed a RSM-containing ration for several weeks were selected for the study. After killing, the birds' caeca were removed, sealed in pairs in plastic bags, frozen with dry ice and stored at -40°C until the contents were used for an inoculum.

The basic medium used in the studies contained: yeast extract, 0.1 g; volatile fatty acid mixture (Caldwell and Bryant, 1966), 0.3 ml; resazurin (1 mg/ml), 0.1 ml; hemin (1 mg/ml), 0.1 ml; Na_2CO_3, 0.4 g; salt solution (Hungate, 1968) 30 ml and distilled water to a volume of 85 ml. After boiling, oxygen free CO_2 was bubbled through the basic medium and 8.5 ml aliquots were transferred to Hungate tubes, stoppered and autoclaved at 121°C for 10 minutes. To each tube of basic medium 0.5 ml of a filter-sterilized (average filter pore size, 0.22 um) solution containing 10 g/100 ml $NaHCO_3$ and 300 mg/100 ml $Na_2S_2O_4$ was added. The final pH of the buffered basic medium was 6.8.

The chemicals added in the various treatments, Table 1, were sterilized in an autoclave at 121°C for 10 min and 0.1 ml aliquots were added to replicate Hungate tubes containing buffered basic medium. The sinapine used in the studies was extracted from rapeseed meal as sinapine bisulfate using the method followed by Clandinin (1961).

Three incubations were undertaken. Each involved inoculum prepared from the caecal contents of a different bird. Inoculum from a bird was prepared by thawing its pair of caeca under N_2 and making the contents of same to 50 ml with sterile 0.1 M phosphate buffer pH 6.8. In each incubation 1.0/ ml of inoculum was added to each replicated Hungate treatment tube. The Hungate tubes were then incubated in a waterbath at 41°C on a rotary shaker (200 rpm, eccentricity, 1"). After 0 and 21 hours of incubation, 1 ml samples of the incubation mixture were withdrawn by sterile syringe and transferred to Vacutainer tubes (Beckton-Dickinson, Rutherford, N.J.) which were then frozen with dry ice and held at -17°C until analysed (within 3 days for birds 1 and 2; 6 days for bird 3).

Analysis of the contents of the Vacutainer tubes was conducted as follows; the frozen samples in the tubes were placed in liquid N_2 to about one-half their height, and, after 15 min. were evacuated on a high vacuum system through a 26 gauge needle for 10 sec. When 3 ml Vacutainers were used (inocula 1 and 2) 1 ml of 50% KOH was injected into the evacuated tubes and when 5 ml Vacutainers were used (inoculum 3) 2 ml of 50% KOH was injected. One ml standards of various concentrations of TMA.HCL received similar KOH treatment. After the samples reached room temperature they were vigorously shaken for 30 sec. and permitted to remain motionless for 5 min. One-half ml of the head-space gas was injected into a gas chromatograph column (Mehta et al., 1974). The column was 3.5 m x 2 mm (ID) glass tubing packed with 40% Penwalt 231 on 80-100 mesh Chromosorb W. (Applied Science Laboratories, State College, Pa.). The gas chromatograph was a Bendix 2500 equipped with a flame ionization detector. Oven temperature was 75°C, carrier gas flow was 40 ml N_2/min. An Autolab Minigrator was used to determine retention times and peak areas. The TMA and DMS peaks were identified by comparison with retention times of standards. The data obtained after 21 hours of incubation were analysed by analysis of variance and differences among means were determined using Duncan' s multiple range test (Steele and Torrie, 1960) at the 5% level of propability.

RESULTS AND DISCUSSION

The results of TMA production from the incubation studies (Table 1) indicated that sinapine bisulfate and choline were converted to TMA by inoculum taken from the caeca of the chickens (2 to 5 vs 1) and that sinapine bisulfate was converted at a slower rate to TMA than choline (2 vs 5). When the bacteria in the inoculum were destroyed by boiling no TMA was produced from the sinapine bisulfate added to the tubes. The results also showed that neither sulphate nor the sinapic acid moiety of sinapine affected the amount of TMA produced.

It will also be noted from Table 1 that production of DMS occurred in the treatments containing sinapine bisulfate (2, 3 and 4) but not in any of the other treatments. It was thought that the sulfate moiety of sinapine bisulfate was the substrate from which the caecal bacteria produced DMS but this opinion was not confirmed by the results of treatments 8 and 9 where sulfate, added as potassium sulfate, did not give rise to DMS.

TABLE 1

TRIMETHYLAMINE AND DIMETHYLSULPHIDE PRODUCTION

Treatments[1]	TMA,µg/ml medium[2]		DMS,µg/ml medium[3]	
	0 hr.	21 hr.	0 hr.	21 hr.
1. Control (C)	0.4	10.8[b]	0	0
2. C + 2 mg sinapine	0.3	28.6[c]	0	5,0[a]
3. C + 10 mg sinapine	0,4	102.4[d]	0	28.6[c]
4. C + 50 mg sinapine	0.4	141.6[e]	0	22.4[b]
5. C + 3.43 mg choline	0.5	142.7[e]	0	0
6. C + 2.35 TMA.HCl	130.0	135.9[e]	0	0
7. C + 10 mg sinapine, boiled	0.4	0.7[a]	0	0
8. C + 3.43 mg choline + 4.28 mg K_2SO_4	0.1	155.3[f]	0	0
9. C + 5.5 mg sinapic acid + 4.28 mg K_2SO_4	0	4.9[4]	0	0

[1]Sinapine was supplied as sinapine bisulphate, choline as choline chloride. Levels of choline chloride, TMA.HCl, K_2SO_4 and sinapic acid used were based on molar equivalents from 10 mg sinapine bisulphate.

[2]Based on inoculum from three birds.

[3]Based on inoculum from two birds.

[4]Based on inoculum from one bird.

The results ot this study lead to the conclusion that bacteria are present in the caeca of chickens which are capable of producing TMA and DMS from sinapic bisulfate prepared from rapeseed meal. The finding may also account for the reason why bad breath in chickens fed RSM has been associated with the laying of fishy eggs by some brown-egg layers.

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