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International Fishmeal & Oil Manufacturers Association

RING TEST FOR DETERMINATION OF BIOGENIC AMINES IN FISH MEAL

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Purpose

The purpose of the ring test is to establish a recommended IFOMA method for measuring biogenic amines in fish meal.

Introduction

Differences in results of biogenic amine analysis on the same sample of fish meal, are thought to be due principally (a) to different methods of extraction of the biogenic amines from the meal and/or (b) to the use of different post-extraction procedures.

Thus, the ring test proposal was written in two parts. The first part was designed to establish the variability that existed between different laboratories using their own methods and to determine whether part of this variation was due to extraction or post-extraction procedures. The second part was a ring test of a draft recommend analytical procedure which was based on the results of the first part.

PART 1

Method

Part 1a

IFOMA collected six fish meal samples, two from each of Peru, Chile and Denmark. From each origin, one sample of the meal was prepared from medium fresh raw material and the other from fresh raw material. Each sample was about 2 kilograms. The samples were sent directly to Laboratory A.

Laboratory A made extracts of each meal using their own method, and in addition, prepared sub-samples of the six meals.

All participating laboratories (Appendix I) received from lab A the following:-

Six extracts of the six fish meals coded M, A, C, F, P, J.

One duplicate extract of J spiked with a known quantity of Histamine, coded S.

One standard (Tyramine, Putrescine, Cadaverine, Histamine, Agmatine) coded ST containing 238, 391, 298, 298, 151µmoles/l respectively.

Each laboratory analysed the extracts for as many biogenic amines as they were able using the post-extraction procedure usual in their laboratory. Not all laboratories have reported the methods used.

All analyses were conducted and reported in duplicate.

Part 1b

Three laboratories, L, C and A received sub-samples of the six meals (50g).
Each laboratory undertook the extraction procedure (in duplicate) usual in their own laboratory and sent a volume of each extract to Lab A equivalent to 1g of fish meal. The extraction procedures were 5g meal extracted with 150 ml water (lab L), 1g meal extracted with 10 ml 10% TCA (lab C) or 10 g meal extracted with 90 ml 10% TCA (lab A). Lab A analysed the content of biogenic amines in all the extracts by its usual procedure of fluorescence detection of post-column OPA derivatives using an external standard for calibration. In addition labs L and C analysed their own extracts by their own normal procedure. For Lab L this was measuring dansyl derivatives in the UV at 254 nm in conjunction with an internal standard. Lab C also used dansyl derivatives measured in the UV at 254 nm but calibrated against an external standard.

Results

Data Set 1a

Eleven laboratories returned data. A preliminary analysis revealed that results from four laboratories (E, G, J, K) were clearly different from the rest. Their data were discarded as outliers.

The data (Appendix Tables 1-5) were derived from a maximum of seven laboratories which provided determinations of five amines from eight samples. For the deviations from the sample mean, the consistency of the signs (+ or -) in any line (lab) give an indication of bias for that laboratories performance. The magnitude of the deviations indicate the size of the bias or large inconsistency. Only 3 laboratories returned results for agmatine. Consistent low bias is seen for Histamine (Lab B), Cadaverine (Lab H), Putrescine (Lab B, H). Consistent high bias is seen for Cadaverine (Lab C, D), Putrescine (Lab C, D). In addition several very large deviations can be seen indicating inconsistency.

The analysis is summarised in Table 1.

The first line of each set (amine) displays the mean determination for each of the eight samples, as derived from the first reported value 'A' of the open duplicates. The second line contains the ‘Between Laboratory’ standard deviation. The third line expresses the previous figure (standard deviation) as a percentage of the mean value. This statistic is often referred to as the coefficient of variation. The fourth line displays the standard deviation as derived from the open duplicates - the so-called “within laboratory” variations.

Since sample S was the same as sample J but with an additional concentration of Histamine, these samples provided an estimate of the hidden duplicate variation for amines other than Histamine. The line labelled Hidden Dup. provides the relevant figures, pooled over laboratory and is probably a more realistic assessment of “within laboratory variations”.

Sample ST contained a specified concentration of the five amines, so that the analysis provided an estimate of bias, and this figure is shown as the last line of each group.

The very large coefficients of variation for the 'Between Laboratory' error were usually from samples of low concentrations, and where there was, of course, substantial error of determination.

The little information that was available on 'Hidden Duplicates' confirmed the customary result that the standard deviation was greater than the corresponding open duplicate figure. The unreliability of within laboratory standard deviation (repeatability) based on open duplicates has been emphasised in the reports of previous ring tests conducted by IFOMA on a variety of measurements.

Although the estimate of bias did not attain statistical significance for any of the amines, it was in fact very close to the significance level for the Tyramine data. Also, it may be noted that for Cadaverine, Tyramine and Putrescine, six out of the seven laboratories returned a determination which was greater than the correct figure.

Figures 1 - 4 show the Principal Components Analysis for each biogenic amine. For Histamine laboratories B and I appear to be different from the other laboratories. For Cadaverine and Tyramine, laboratories B, C and D seem to differ from A, F, H and I. For Putrescine laboratories D and I seem to be the most divergent.

Data Set 1b

The intention here was to assess the degree of variation due to the use of different extraction procedures, and that due to the analysis. However, since there were only five independent observations for each sample in an unbalanced design, the relevant statistics were based on quite small amounts of information. Ideally, there should be a balanced arrangement over numerous laboratories. The present design is illustrated below, *a, b, c, d, e* indicating a data point.

			Extraction	
	Lab	A	C	L
	A	*a*	*b*	*c*
Analysis	C		*d*	
	L			*e*

The four degrees of freedom (df) among five observations can provide estimates of the 'Extraction' standard deviation, and the 'Analysis' standard deviation. The extraction error term is estimated with 2 degrees of freedom for each sample from analysis at Lab A only i.e. comparing values *a, b, c*. The 'Analysis' standard deviation with 2 df is based on the comparison of the analysis in Lab C with that in Lab A and Lab L of the same extract i.e. (*b-d*) + (*c-e*). In effect, the 'Analysis' variation is also a reflection of the difference between using OPA (with fluorescence detection) and dansyl (with UV detection) derivatives of the amines. Table 2 summarises the result of this exercise.

The standard deviations displayed in Table 2 suggest that the errors of analysis are rather greater than those of extraction. This was not invariably the case; however, it should be noted

how frequently the entry on the third line is greater than that on the second line. The data was too sparse to provide the 'Analysis' errors for Agmatine and Tyramine.

Discussion

Part 1a indicated considerable variation between laboratories in their ability to determine biogenic amines and especially histamine in circulated extracts of fish meals. Even when 4 out of 11 laboratories were discarded as outliers the coefficient of variations were very high. Table 3a gives the average CV for determinations within ranges <20, >20<200, and >200 umole/litre. Within the range 20-200 the CV averaged 25-26% for histamine and tyramine, and was even worse for cadaverine (36%) and putrescine (45%). Agmatine, an amine that decreases with deterioration of raw fish and therefore is of little use as a screen for freshness, was the only one approaching reasonable reproducibility at 12% CV. The above ranges correspond approximately to <40, 40-400, and 400-4000 ppm of fish meal. However, the different methods had different sensitivities depending on whether UV or fluorescence was used and the extracts presumably were diluted differently according to the method. If not, part of the variation may be due to inappropriate concentration, either very low or very high, in the different methods.

Similar values for the variation in analysis of the same extract were obtained in Part 1b (Table 3b). As noted previously, this variation could be due to comparison of essentially two very different methods with different reagents, sensitivity and method of detection. In contrast, the methods of extraction did not differ so greatly. Consequently, it seems reasonable that the variation due to method of analysis was generally greater than that due to extraction.

However, even the different extracts analysed in the same laboratory had a high variability, the mean coefficiant of variations in the range 10-20% for moderate or high samples and even greater for samples with low values (Table 3c). This variation includes the within laboratory variation. The experimental design did not permit separation of the components of within laboratory variation and that due to extraction per se.

Conclusions

1. There is an unacceptably large variation between laboratories in analysis of the same extract of fish meal or of a standard solution of biogenic amines.

2. The results suggest that the errors of analysis are rather greater than those of extraction. This may be due to the use of very disparate methods of analysis.

3. Variation due to extraction method is also appreciable.

4. A single well defined method, defining both extraction and analysis, is needed for commercial contracts.

5. IFOMA should test and recommend such a method.

It was therefore decided to adopt the Torry method as the draft recommended analytical method to be tested in Part 2.

Table 1. Table summarising the analyses of Data Set 1a. (µmoles/litres)

	Sample Code							
	M	A	C	F	P	J	S	ST
Histamine								
Mean Value	37.17	482.81	76.16	1006.69	12.86	16.4	311.93	298.73
St. Dev.	8.83	149.98	18.74	125.67	21.81	19.89	44.05	30.71
Coeff. Var.	23.74	31.06	24.61	12.48	169.66	121.28	14.12	10.28
Open Dup.	4.44	18.74	8.18	19.79	7.35	2.89	13.31	26.73
Bias								0.73 ±11.6
Cadaverine								
Mean Value	33.07	180.89	83.3	452.44	1203.87	130.13	128.82	337.41
St. Dev.	27.96	75.37	30.42	92.47	362.24	46.31	37.57	54.62
Coeff. Var.	84.54	41.67	36.52	20.44	30.09	35.58	29.16	16.19
Open Dup.	1.7	5.1	8.64	18.64	45.6	5.39	6.99	33.93
Hidden Dup.							17.91	
Bias								39.4 ±20.6
Tyramine								
Mean Value	8.86	48.31	17.63	121.1	366.36	27.89	27.47	255.87
St. Dev.	14.9	17.32	6.29	28.22	49.11	5.42	7.36	18.65
Coeff. Var.	168.28	35.85	35.67	23.3	13.4	19.43	26.8	7.29
Open Dup.	2.1	4.53	1.54	9.1	27.82	1.84	2.05	18.1
Hidden Dup.							5.03	
Bias								17.9 ±7.1
Putrescine								
Mean Value	21.51	71.16	74.8	309.36	814.27	57.46	60.98	466.31
St. Dev.	15.45	50.26	36.2	117.26	327.08	25.87	25.46	108.71
Coeff. Var.	71.81	70.63	48.4	37.9	40.17	45.02	41.75	23.31
Open Dup.	3.24	6.96	1.66	10.35	19.87	2.67	3.91	35.68
Hidden Dup.							6.3	
Bias								75.3 ±41.1
Agmatine								
Mean Value	21.67	93.67	22.33	55.33	80.67	41.33	39.33	150
St. Dev.	4.04	13.05	3.21	4.73	13.43	1.53	5.86	17.78
Coeff. Var.	18.65	13.93	14.39	8.54	16.65	3.7	14.9	11.85
Open Dup.	4.08	2.42	0	2.08	0.41	3.29	2.12	2.86
Hidden Dup.							3.79	
Bias								-1.0 ±10.3

Table 2. Table summarising the analyses of Data Set 1b. The row headed Est. is the determination from Lab A. The rows headed Extr. and Anal. display the Standard Deviations of 'Extraction' and 'Analysis'. The figures in brackets express the standard deviations as percentages of the determination in Lab A. (Measurements in mg / kg fish meal)

	Sample Code					
	M	A	C	F	J	P
Histamine						
Est.	78	1189	144	2212	44	22
Extr.	12.7 (16.2)	141.8 (11.9)	33.5 (23.3)	205.3 (9.3)	3.1 (6.9)	8.2 (37.2)
Anal.	24.8 (31.8)	165.3 (13.9)	15.8 (11.0)	161.3 (7.3)	28.9 (65.7)	47.6 (217)
Cadaverine						
Est.	51	399	133	920	225	2626
Extr.	7.6 (15.0)	45.0 (11.3)	38.6 (29.0)	84.4 (9.2)	37.0 (16.4)	258.5 (9.8)
Anal.	32.9 (64.4)	91.6 (23.0)	11.3 (8.5)	90.4 (9.8)	25.6 (11.4)	274.6 (10.5)
Agmatine						
Est.	52	273	65	156	104	234
Extr.	11.8 (22.7)	49.5 (18.1)	14.9 (22.9)	21.8 (14.0)	37.5 (36.1)	35.9 (15.3)
Tyramine						
Est.	14	165	41	384	82	1043
Extr.	11.8 (84.2)	19.2 (11.6)	50.3 (12.3)	29.0 (7.6)	15.5 (18.9)	92.5 (8.87)
Putrescine						
Est.	26	115	97	494	79	1305
Extr.	4.0 (15.5)	16.5 (14.4)	10.6 (10.9)	48.7 (9.9)	24.4 (30.9)	132.0 (10.1)
Anal.	41.3 (159)	24.8 (21.6)	17.4 (17.9)	73.8 (14.9)	34.8 (44.1)	133.9 (10.3)

Table 3. Summary of Coefficient of Variation according to concentration of amines

Table 3a. Data from Data Set 1a. Average of CV values of 8 extracts or standards analysed in 7 laboratories using different methods

	Histamine	Cadaverine	Tyramine	Putrescine	Agmatine
Values <20 uM	145	No values	36	No values	No values
Values >20<200 uM	25	36	26	45	12
Values >200<2000 uM	17	22	10	43	No values

Table 3b. Data from Data Set 1b. Average values of CV of 6 meals extracted in duplicate in three laboratories and analysed by one laboratory (Extraction)

	Histamine	Cadaverine	Tyramine	Putrescine	Agmatine
Values <40 ppm	37	No values	84	16	No values
Values >40<400 ppm	15	18	13	19	22
Values >400<4000 ppm	11	10	9	10	No values

Table 3c. Data from Data Set 1b. Average of CV values of 12 extracts analysed in three laboratories (Analysis)

	Histamine	Cadaverine	Tyramine	Putrescine	Agmatine
Values <40 ppm	217	No values	No data	159	No data
Values >40<400 ppm	36	27	No data	28	No data
Values >400<4000 ppm	11	10	No data	13	No data

PART 2

Method (Part 2)

The draft recommended analytical method (Torry method - Appendix 2) was sent to participating laboratories for comment.

Ten laboratories agreed to participate in Part 2. A list of those laboratories is given in Appendix 3. Each participating laboratory received twelve samples of fish meal for determination of biogenic amines in duplicate according to the method in Appendix 2. The samples were prepared and distributed by Fish Industries from original samples obtained from: Denmark, Norway, Chile and Peru

The same samples were used in the ring test on pepsin digestibility (see Research Report 2000-1). Samples one and five were hidden duplicates. Samples three and ten were a different set of hidden duplicates.

All analysis were conducted and reported in duplicate.

Not all participating laboratories used the proposed method as shown in Appendix 2. The key features of the proposed method are extraction of 15 g sample with 150 ml 0.6M perchloracetic acid, addition of an internal standard of 1,6-diamino hexane, separation on a HPLC 150 x 4.6 mm column of ODS, post-column derivitisation with OPA and measurement of fluorescence with excitation at 365 nm and emission at 418 nm. Some laboratories introduced variations which are listed below.

Laboratory 9 used their ususal method which differed completely, using 10% TCA for extraction, precolumn derivitisation with dansyl chloride and detection in UV at 254 nm.

Laboratory 2 also used their usual method with extraction with 10% trichloracetic acid and precolumn instead of post column derivatisation with OPA. A further difference was the extraction of 5 g samples in 50 ml extraction media. Laboratory 2 used a 5 µm Hypersil BDS C18 column 250mm x 4mm and eluted with disodium phosphate-acetonitrile instead of acetate- acetonitrile-methanol solvents. Laboratory 2 used excitation 350 nm and emission 450 nm.

Laboratory 4 also used precolumn instead of post column derivatisation with OPA.

Laboratory 7 homogenised 5 g samples in 50 ml extraction media instead of 15 g in 150 ml. Also it used excitation 345 nm and emission 455 nm.

The above differences were considered to be sufficiently great to warrant exclusion of Laboratories 2, 4 and 9 from the results for the final analysis of the repeatability and reproducibility of the circulated method. This reduced the number of accepted laboratories to 7 even before scrutiny of the results. ISO 5725-1 refers to the use of 8 to 15 laboratories and

IUPAC gives a minimum of 8 laboratories. Consequently, there were insufficient laboratories following the prescribed method to give an acceptable collaborative study.

Results (Part 2)

Appendices tables 6 - 10 give for each laboratory and sample the deviations from the meal mean value. In this tabulation the first reported or 'a' value only is used. The consistency of the signs (+ or -) give an indication of bias for that laboratories performance. The magnitude of the deviations indicate the size of the bias or large inconsistency. The final column of each appendix table gives the arithmetic mean value over all laboratories. Only 5 laboratories returned results for agmatine.

The consistent negative signs for Histamine (Lab 10), Tyramine (Labs 4, 9), Putrescine (Labs 8, 10), Cadaverine (Labs 8, 10) and Agmatine (Lab 8)) indicate labs with consistently low values. The consistent positive signs for Tyramine (Lab 6) Cadaverine (Labs 3, 5) and Agmatine, (Lab 4) indicate labs with consistently high values. There are often large deviations e.g. Lab 4 for all amines. These may be in the same direction as the general bias (e.g. Tyramine, Labs 4, 9; Putrescine, Lab 10; Cadaverine, Lab 10; Agmatine, Lab 4) or in the opposite direction (e.g. Histamine, Labs 1, 6, 9) indicating great variability.

Table 4 gives the laboratory mean values over all twenty-four observations on the twelve samples of fish meal. The standard deviation (SD reps) between open replicates ('a' and 'b' values) and this value expressed as a % of the mean (CV %) are also given. Using the 'a' values only, the standard deviation between hidden duplicate samples (SD dups) and this value expressed as a % of the mean of the four samples is given.

The first point of note is the low mean value averaged over all samples and laboratories for the biogenic amines and especially histamine. This reflects that many of the meals were of high quality, presumably prepared from fresh raw material, in which the amount of histamine was very low and in several cases below the detection limit of the laboratories. (See Table 5 for the meal mean values for histamine.) For such samples the estimate of variability is a false zero. This is particularly important with respect to the hidden duplicate pair of meals 1 and 5 which six labs reported as having histamine below the limit of detection. With samples varying from virtually zero to a few with moderately high contents it is not possible to combine the estimates to give pooled estimates of within and between laboratory variability.

Secondly, the SD and CV based on the hidden duplicates is on average 2 to 5 times greater than that based on the open replicates and in several individual cases many times greater. In the case of histidine the between hidden duplicate variability is also severely underestimated because of the 6 zero values for variability of the pair meals 1 and 5 reported as below detection limits. As in earlier collaborative studies the use of open duplicates greatly underestimates the true within laboratory variability.

Thirdly, there are some very large values for SD dups, indicating poor consistency of determination between the hidden duplicates, particularly for laboratory four. Using Cochran test of homogeneity of within laboratory variances based on the hidden duplicates, laboratory 4 is identified as an outlier for histamine, putrescine and cadaverine (compare values for SD dups between Laboratories). It also has the highest SD (dups) for tyramine although this did not reach the critical significance level. Lab 4 also had high variability between the open

duplicates, particularly for histamine and cadaverine where the SD reps was far greater than any other laboratory. In addition, Lab 4 had the highest value for histamine and agmatine and was an outlier by the Grubbs test for both amines. After eliminating Lab 4, Lab 1 was also an outlier by the Cochran test for histamine, putrescine and cadaverine (second highest SD dups).

Tests for outlier labs were also carried out starting with labs which apparently followed the circulated procedure (i.e. omitting labs 2, 4, 9). Again Lab 1 was identified as an outlier by the Cochran test for histamine, putrescine and cadaverine. Although determinations by Lab 1 were close to the overall mean the hidden duplicate results differed markedly, except for the pair 1 and 5 for histamine where both were recorded as <0.5 mg/kg. For example, for histamine the other hidden pair 3 and 10 were reported as 579 and <0.5 mg/kg. No other laboratory reported such extreme values for either meal 3 or 10, with the mean values of 108 and 123 mg / kg respectively over the remaining laboratories (excluding Lab 4). Indeed the high value for meal 3 was identified as an outlier by the Grubbs test but the low value was not. Laboratory 1 also reported values of <0.5 mg /kg for histamine in meals 6, 8 and 9 whereas the means of the remaining labs were 20, 171 and 11 for these meals respectively. Such complete absence of a discernible peak suggest either the wrong sample or misidentification of the histamine peak on the chromatogram.

Tables 5-10 give the meal mean values together with the standard deviation (SD) between laboratories and this value expressed as the Coefficient of Variation (CV). The values are calculated both for all the laboratories and also after omitting labs 1, 2, 4 and 9 either as outliers or because they did not use the prescribed method. Omitting the selected laboratories produced an average decrease of 53% in CV for histamine, 28% for tyramine but had very little effect for putrescine (3% less) or cadaverine (5% less). Omitting Lab 4 from the limited agmatine data reduced average CV by 67%. Even so the CV remained unacceptably high with values in the range 20 - 40 % for meals with 40 to 400 ppm amine. For values <40 ppm the CV was even greater but for values >400 ppm there was no apparent improvement.

	Histamine	Tyramine	Putrescine	Cadaverine	Agmatine
<40 ppm	72	92	74	No values	54
40<400 ppm	37	22	41	34	29
>400 ppm	15	31	38	35	40

Conclusions

1. Insufficient laboratories followed the prescribed analytical method to give a valid test of method reproducibility.

2. The meals distributed were inappropriate for the measurement of biogenic amines, especially for histamine, as the majority had very low values indicating freshness of raw material.

3. The estimate of within laboratory variation from hidden duplicates was much greater than that estimated from the open replicates. This was so despite the under-estimate of the hidden

duplicate variation because one pair of meals had histamine levels below the detection limit for many laboratories.

4. The variation between laboratories analysing the same samples is extremely great. Part of this difference may be due to the use of different methods. Even when only data from laboratories using the same method and after the removal of outliers, the variation is unacceptably great. This has implications for contracts specifying levels of biogenic amines.

5. Laboratories identified as outliers must examine their technique to establish the cause of variation prior to participating in further ring tests.

6. The best method for extraction and analysis should be reviewed in the light of other recent work and collaborative studies. The collaborative study with fish meal should be repeated using samples covering the full range of expected values and with laboratories restricted to those who can under take the prescribed method and are routinely using the method for quality control work related to the fish meal industry.

Table 4. Laboratory means and within laboratory standard deviation (SD) and coefficient of variation (CV) measured from open replicates and hidden duplicates.
(Units mg / kg fish meal)

	Laboratory Code										
	1	2	3	4	5	6	7	8	9	10	Mean
Histamine											
All samples											
Mean	144	119	146	355	117	169	142	90	136	104	152
SD reps	29.8	9.5	5.4	76.6	3.6	27.6	8.0	16.0	5.2	6.0	
CV (%)	20.7	8.0	3.7	21.6	3.1	16.4	5.6	17.6	3.9	5.8	11
Between hidden duplicates (using 'a' values only)											
Mean	145	62	78	308	64	122	73	58	66	41	102
SD dups	289.5	3.5	5.0	390.0	34.8	83.5	3.0	1.1	8.5	6.0	
CV (%)	199.3	5.6	6.5	126.8	54.5	68.6	4.2	1.9	13.0	14.8	50
Tyramine											
All samples											
Mean	377	340	378	135	338	423	439	387	92	186	309
SD reps	31.1	25.2	22.4	37.6	18.9	71.7	9.8	25.2	14.0	11.6	
CV (%)	8.2	7.4	5.9	27.9	5.6	17.0	2.2	6.5	15.3	6.2	10
Between hidden duplicates (using 'a' values only)											
Mean	867	864	890	308	834	991	1058	953	168	335	727
SD dups	215.2	52.6	118.5	246.2	244.0	153.5	51.5	69.1	12.1	41.7	
CV (%)	24.8	6.1	13.3	80.1	29.3	15.5	4.9	7.2	7.2	12.5	20
Putrescine											
All samples											
Mean	335	382	350	214	378	365	322	167	335	116	296
SD reps	92.7	20.8	15.8	82.4	7.2	33.8	16.0	23.4	23.9	11.5	
CV (%)	27.7	5.4	4.5	38.5	1.9	9.2	5.0	14.1	7.1	9.9	12
Between hidden duplicates (using 'a' values only)											
Mean	686	845	753	260	843	772	708	416	695	197	617
SD dups	265.8	49.0	96.6	390.1	35.5	80.8	8.7	20.7	19.1	14.7	
CV (%)	38.7	5.8	12.8	150.0	4.2	10.5	1.2	5.0	2.8	7.5	24
Cadaverine											
All samples											
Mean	729	939	1028	452	1204	948	977	565	955	320	812
SD reps	77.2	60.1	44.2	207.9	170.2	64.5	33.9	58.9	43.6	18.7	
CV (%)	10.6	6.4	4.3	46.0	14.1	6.8	3.5	10.4	4.6	5.8	11
Between hidden duplicates (using 'a' values only)											
Mean	1329	2076	2268	713	2607	1990	2157	1200	2089	535	1696
SD dups	452.6	169.0	277.4	1058.8	96.0	182.5	106.8	51.2	45.5	30.7	
CV (%)	34.0	8.1	12.2	148.6	3.7	9.2	5.0	4.3	2.2	5.7	23
Agmatine											
All samples											
Mean	*	*	224	1031	197	*	267	180	*	*	380
SD reps	*	*	16.1	226.0	7.4	*	8.6	28.0	*	*	
CV (%)	*	*	7.2	21.9	3.8	*	3.2	15.6	*	*	10
Between hidden duplicates (using 'a' values only)											
Mean	*	*	328	1313	383	*	483	361	*	*	573

SD dups	*	*	376.2	276.8	155.3	*	39.0	44.4	*	*	
CV (%)	*	*	114.9	21.1	40.6	*	8.1	12.3	*	*	39

Table 5. Histamine. Mean values for each meal with between laboratory standard deviation (SD) and coefficient of variation (CV) for data from all laboratories and after exclusion of labs 1, 2, 4, 9. (Units mg/ kg meal; based on 'a' values only.)

	All labs			Excluding labs 1, 2, 4, 9		
Meal	Mean	SD	CV	Mean	SD	CV
1	113	269.7	238.8	30	19.1	64.2
2	662	91.9	13.9	647	101.9	15.8
3	154	143.5	93.4	108	24.9	23.2
4	167	45.3	27.2	148	38.4	25.9
5	33	39.9	120.0	27	19.1	70.6
6	80	187.4	233.7	26	17.3	65.7
7	44	34.7	78.9	34	15.1	45.0
8	155	61.2	39.4	167	41.3	24.8
9	103	279.3	270.6	15	16.9	112.8
10	106	69.1	65.0	125	74.7	59.6
11	109	31.7	29.1	118	29.9	25.4
12	89	47.9	54.0	96	59.1	61.7

Table 6. Tyramine. Mean values for each meal with between laboratory standard deviation (SD) and coefficient of variation (CV) for data from all laboratories and after exclusion of labs 1, 2, 4, 9. (Units mg/ kg meal; based on 'a' values only.)

	All labs			Excluding labs 1, 2, 4, 9		
Meal	Mean	SD	CV	Mean	SD	CV
1	699	241.8	34.6	751	216.3	28.8
2	56	12.9	23.0	63	8.7	13.9
3	810	394.1	48.7	1002	284.4	28.4
4	38	19.8	51.4	47	21.0	44.9
5	620	278.0	44.8	720	238.4	33.1
6	104	23.9	23.0	113	23.5	20.8
7	229	73.0	31.9	254	61.1	24.1
8	23	23.7	101.9	29	26.9	92.2
9	86	35.7	41.4	99	20.3	20.5
10	777	399.2	51.4	901	285.7	31.7
11	171	43.3	25.3	190	18.4	9.7
12	110	31.5	28.7	128	23.4	18.3

Table 7. Putrescine. Mean values for each meal with between laboratory standard deviation (SD) and coefficient of variation (CV) for data from all laboratories and after exclusion of labs 1, 2, 4, 9. (Units mg/ kg meal; based on 'a' values only.)

	All labs			Excluding labs 1, 2, 4, 9		
Meal	Mean	SD	CV	Mean	SD	CV
1	716	213.0	29.7	608	211.3	34.8
2	52	23.5	44.8	44	22.6	51.6
3	548	273.9	50.0	612	250.6	41.0
4	56	26.9	47.7	49	26.0	52.6
5	610	256.0	42.0	620	206.6	33.3
6	91	33.4	36.7	90	35.2	39.2
7	292	114.2	39.1	253	90.7	35.9
8	41	29.7	72.7	20	15.1	74.0
9	205	96.2	46.9	151	60.6	40.0
10	595	284.8	47.9	619	268.8	43.4
11	236	73.3	31.1	199	55.7	28.0
12	118	45.7	38.6	118	45.5	38.6

Table 8. Cadaverine. Mean values for each meal with between laboratory standard deviation (SD) and coefficient of variation (CV) for data from all laboratories and after exclusion of labs 1, 2, 4, 9. (Units mg/ kg meal; based on 'a' values only.)

	All labs			Excluding labs 1, 2, 4, 9		
Meal	Mean	SD	CV	Mean	SD	CV
1	1684	558.7	33.2	1546	608.0	39.3
2	212	58.5	27.6	196	64.3	32.7
3	1782	925.7	51.9	2047	858.8	42.0
4	250	72.2	28.9	239	77.3	32.3
5	1418	632.0	44.6	1475	512.0	34.7
6	319	86.6	27.1	317	97.3	30.7
7	779	219.3	28.1	736	232.0	31.5
8	70	30.0	43.1	68	32.1	47.5
9	386	205.7	53.4	341	125.2	36.7
10	1901	868.7	45.7	2104	888.3	42.2
11	512	111.6	21.8	505	91.5	18.1
12	380	94.2	24.8	383	83.9	21.9

Table 9. Agmatine. Mean values for each meal with between laboratory standard deviation (SD) and coefficient of variation (CV) for data from all laboratories and after exclusion of labs 1, 2, 4, 9. (Units mg/ kg meal; based on 'a' values only.)

	All labs			Excluding labs 1, 2, 4, 9		
Meal	Mean	SD	CV	Mean	SD	CV
1	462	424.1	91.8	250	11.6	4.6
2	121	175.0	144.6	34	15.9	47.1
3	633	348.7	55.0	509	273.6	53.7
4	305	349.9	114.6	132	47.9	36.4
5	514	663.3	129.0	183	27.0	14.8
6	213	249.1	117.0	89	18.7	21.0
7	626	490.6	78.4	382	66.2	17.3
8	344	623.2	181.1	33	19.6	59.9
9	516	629.1	122.0	202	55.7	27.6
10	684	200.1	29.2	613	156.6	25.6
11	173	197.1	113.7	77	42.9	56.0
12	230	246.2	106.9	110	62.0	56.2

Part 1. Appendix Tables 1-5

Tables giving 'A' determinations, and deviations from the sample means.
(Units µmoles/litre)

Appendix Table 1. Histamine

				Sample				
Lab.	M	A	C	F	P	J	S	ST
A	36.00	540.00	67.00	974.00	11.00	21.00	327.00	305.00
B	28.00	358.00	64.00	785.00	0.00	10.00	*	291.00
C	40.50	620.80	81.00	1199.30	0.00	0.00	353.60	351.80
D	38.00	529.00	62.00	959.00	11.00	0.00	294.00	285.00
F	51.00	589.00	74.00	1027.00	7.00	0.00	342.00	252.00
H	42.00	543.00	69.00	1071.00	61.00	52.00	323.00	316.00
I	24.70	199.90	116.10	1031.50	0.00	31.80	232.00	290.30
Mean Values	37.17	482.81	76.16	1006.69	12.86	16.40	311.93	298.73
Deviations from the Mean Values								
A	-1.17	57.19	-9.16	-32.69	-1.86	4.6	15.07	6.27
B	-9.17	-124.81	-12.16	-221.69	-12.86	-6.4	*	-7.73
C	3.33	137.99	4.84	192.61	-12.86	-16.4	41.67	53.07
D	0.83	46.19	-14.16	-47.69	-1.86	-16.4	-17.93	-13.73
F	13.83	106.19	-2.16	20.31	-5.86	-16.4	30.07	-46.73
H	4.83	60.19	-7.16	64.31	48.14	35.6	11.07	17.27
I	-12.47	-282.91	39.94	24.81	-12.86	15.4	-79.93	-8.43

Appendix Table 2. Cadaverine

				Sample				
Lab.	M	A	C	F	P	J	S	ST
A	26.00	197.00	67.00	441.00	1366.00	114.00	112.00	305.00
B	13.00	100.00	42.00	287.00	700.00	57.00	*	371.00
C	39.10	261.30	98.80	579.30	1783.80	151.70	153.60	443.20
D	93.00	260.00	120.00	531.00	1433.00	212.00	184.00	341.00
F	27.00	206.00	70.00	472.00	881.00	124.00	137.00	313.00
H	21.00	179.00	64.00	429.00	1089.00	123.00	109.00	284.00
I	12.40	62.90	121.30	427.80	1174.30	129.20	77.30	304.70
Mean Values	33.07	180.89	83.30	452.44	1203.87	130.13	128.82	337.41
Deviations from the Mean Values								
A	-7.07	16.11	-16.3	-11.44	162.13	-16.13	-16.82	-32.41
B	-20.07	-80.89	-41.3	-165.44	-503.87	-73.13	*	33.59
C	6.03	80.41	15.5	126.86	579.93	21.57	24.78	105.79
D	59.93	79.11	36.7	78.56	229.13	81.87	55.18	3.59
F	-6.07	25.11	-13.3	19.56	-322.87	-6.13	8.18	-24.41
H	-12.07	-1.89	-19.3	-23.44	-114.87	-7.13	-19.82	-53.41
I	-20.67	-117.99	38	-24.64	-29.57	-0.93	-51.52	-32.71

Appendix Table 3. Tyramine

	Samples							
Lab.	M	A	C	F	P	J	S	ST
A	5.00	61.00	16.00	137.00	404.00	31.00	31.00	256.00
B	2.00	29.00	9.00	62.70	402.00	16.00	*	243.00
C	0.00	66.30	22.50	142.90	375.40	31.30	36.40	287.20
D	42.00	56.00	26.00	123.00	274.00	28.00	21.00	252.00
F	8.00	57.00	15.00	14.00	391.00	28.00	34.00	270.00
H	5.00	49.00	12.00	126.20	324.00	30.00	24.00	229.00
I	0.00	19.90	22.90	111.90	394.00	30.90	18.40	253.90
Mean Values	8.86	48.31	17.63	102.53	366.34	27.89	27.47	255.87
Deviations from the Mean Values								
A	-3.86	12.69	-1.63	15.9	37.64	3.11	3.53	0.13
B	-6.86	-19.31	-8.63	-58.4	35.64	-11.89	*	-12.87
C	-8.86	17.99	4.87	21.8	9.04	3.41	8.93	31.33
D	33.14	7.69	8.37	1.9	-92.36	0.11	-6.47	-3.87
F	-0.86	8.69	-2.63	22.9	24.64	0.11	6.53	14.13
H	-3.86	0.69	-5.63	5.1	-42.36	2.11	-3.47	-26.87
I	-8.86	-28.41	5.27	-9.2	27.74	3.01	-9.07	-1.97

Appendix Table 4. Putrescine

	Samples							
Lab.	M	A	C	F	P	J	S	ST
A	15.00	66.00	57.00	274.00	787.00	46.00	46.00	405.00
B	7.00	29.00	29.00	151.00	333.00	21.00	*	401.00
C	24.90	86.20	82.80	375.40	1153.30	68.00	69.20	590.80
D	52.00	173.00	128.00	530.00	1319.00	106.00	108.00	653.00
F	28.00	66.00	57.00	287.00	719.00	51.00	59.00	408.00
H	14.00	57.00	53.00	266.00	663.00	51.00	43.00	379.00
I	9.70	20.90	116.80	282.10	725.60	59.20	40.70	427.40
Mean Values	21.51	71.16	74.80	309.36	814.27	57.46	60.98	466.31
Deviations from the Mean Values								
A	-6.51	-5.16	-17.80	-35.36	-27.27	-11.46	-14.98	-61.31
B	-14.51	-42.16	-45.80	-158.36	-481.27	-36.46	*	-65.31
C	3.39	15.04	8.00	66.04	339.03	10.54	8.22	124.49
D	30.49	101.84	53.20	220.64	504.73	48.54	47.02	186.69
F	6.49	-5.16	-17.80	-22.36	-95.27	-6.46	-1.98	-58.31
H	-7.51	-14.16	-21.80	-43.36	-151.27	-6.46	-17.98	-87.31
I	-11.81	-50.26	42.00	-27.26	-88.67	1.74	-20.28	-38.91

Appendix Table 5. Agmatine

	Samples							
Lab.	M	A	C	F	P	J	S	ST
A	21.00	106.00	26.00	59.00	96.00	41.00	46.00	170.00
B	*	*	*	*	*	*	*	*
C	*	*	*	*	*	*	*	*
D	*	*	*	*	*	*	*	*
F	26.00	95.00	20.00	57.00	75.00	43.00	37.00	144.00
H	18.00	80.00	21.00	50.00	71.00	40.00	35.00	136.00
I	*	*	*	*	*	*	*	*
Mean	21.67	93.67	22.33	55.33	80.67	41.33	39.33	150.00
Deviations from Mean Values								
A	0.67	-12.33	-3.67	-3.67	-15.33	0.33	-6.67	-20.00
B	*	*	*	*	*	*	*	*
C	*	*	*	*	*	*	*	*
D	*	*	*	*	*	*	*	*
F	-4.33	-1.33	2.33	-1.67	5.67	-1.67	2.33	6.00
H	3.67	13.67	1.33	5.33	9.67	1.33	4.33	14.00
I	*	*	*	*	*	*	*	*

Part 2.

Appendix Table 6. Histamine. Deviations from the overall meal mean for each laboratory and meal. (Units mg/ kg meal)

	Laboratory Code										
	1	2	3	4	5	6	7	8	9	10	Mean
Meal											
1	-112	-111	-103	807	-75	-63	-58	-107	-85	-93	113
2	-41	29	48	-32	70	-26	101	-194	131	-89	662
3	426	-28	-14	-64	-22	-44	-67	-44	-59	-87	154
4	9	-18	43	93	-80	-28	11	-22	23	-35	167
5	-33	-31	-23	107	-12	17	23	-28	-6	-13	33
6	-80	-78	-40	560	-75	-30	-43	-75	-78	-60	80
7	-29	-2	6	96	-17	6	-11	-39	-3	-8	44
8	-155	31	55	25	-43	65	32	-29	30	-11	155
9	-103	-101	-93	837	-99	-53	-103	-98	-103	-83	103
10	-106	13	44	-26	-42	171	-13	6	6	-51	106
11	32	-26	11	-49	-11	70	15	-14	-10	-17	109
12	10	-38	1	1	-16	136	-16	-26	-15	-39	89

Appendix Table 7. Tyramine. Deviations from the overall meal mean for each laboratory and sample. (Units mg/ kg meal)

	Laboratory Code										
	1	2	3	4	5	6	7	8	9	10	Mean
Meal											
1	114	97	121	-9	183	45	271	97	-515	-405	699
2	8	-4	4	-16	2	1	10	-3	-25	24	56
3	-80	192	200	-590	321	386	408	261	-675	-424	810
4	0	-15	12	-18	-19	12	21	-16	-16	42	38
5	147	83	-10	-410	-78	379	284	305	-418	-279	620
6	9	-28	26	-4	-17	41	26	0	-32	-22	104
7	58	5	-9	-129	-26	132	71	26	-85	-46	229
8	14	-21	-13	-13	-12	27	-6	-16	-15	57	23
9	39	2	34	-46	-14	37	28	1	-74	-6	86
10	380	176	343	-667	4	248	362	244	-626	-460	777
11	44	-4	29	-61	17	12	54	-1	-94	3	171
12	10	-28	20	-40	8	62	25	5	-48	-14	110

Appendix Table 8. Putrescine. Deviations from the overall meal mean for each laboratory and sample. (Units mg/ kg meal)

	Laboratory Code										
	1	2	3	4	5	6	7	8	9	10	Mean
Meal											
1	183	227	84	144	86	-42	0	-288	99	-489	716
2	19	38	18	-12	5	3	3	-37	8	-42	52
3	-140	267	212	-508	289	268	155	-147	-1	-394	548
4	23	41	24	-26	5	3	12	-43	5	-42	56
5	-16	254	10	-530	218	219	115	-150	230	-354	610
6	-10	40	39	-41	21	25	3	-62	19	-33	91
7	-25	-46	-32	258	50	80	-25	-142	50	-167	292
8	41	3	-31	49	-15	9	-19	-36	28	-31	41
9	15	-2	5	205	9	-26	-43	-156	105	-111	205
10	248	162	235	-535	308	175	93	-221	-19	-446	595
11	33	43	4	154	-18	22	-16	-143	-6	-73	236
12	32	28	42	-78	19	43	8	-88	23	-25	118

Appendix Table 9. Cadaverine. Deviations from the overall meal mean for each laboratory and sample. (Units mg/ kg meal)

	Laboratory Code										
	1	2	3	4	5	6	7	8	9	10	Mean
Meal											
1	-455	333	536	566	565	-289	84	-594	387	-1133	1684
2	9	66	28	-32	62	3	19	-96	50	-110	212
3	-788	582	648	-1642	1258	548	878	-519	262	-1226	1782
4	17	45	50	-80	48	18	56	-111	83	-126	250
5	-223	434	312	-1278	643	272	334	-338	729	-884	1418
6	0	25	61	-99	100	80	18	-130	88	-140	319
7	48	-214	11	161	262	115	50	-293	269	-405	779
8	31	4	30	-40	23	20	4	-55	16	-36	70
9	62	-1	124	484	60	10	-53	-190	-281	-219	386
10	-2	169	790	-1581	1179	645	547	-535	194	-1404	1901
11	64	119	18	-222	77	81	39	-171	81	-84	512
12	60	35	50	-190	96	75	13	-77	75	-137	380

Appendix Table 10. Agmatine. Deviations from the overall meal mean for each laboratory and sample. (Units mg/ kg meal)

	Laboratory Code										
	1	2	3	4	5	6	7	8	9	10	Mean
Meal											
1	*	*	-222	848	-196	*	-224	-206	*	*	462
2	*	*	-81	349	-78	*	-75	-115	*	*	121
3	*	*	-563	497	68	*	140	-140	*	*	633
4	*	*	-125	695	-216	*	-126	-227	*	*	305
5	*	*	-334	1326	-362	*	-288	-341	*	*	514
6	*	*	-103	497	-149	*	-110	-135	*	*	213
7	*	*	-186	974	-352	*	-194	-242	*	*	626
8	*	*	-284	1246	-319	*	-305	-338	*	*	344
9	*	*	-246	1254	-370	*	-272	-367	*	*	516
10	*	*	136	286	-272	*	12	-161	*	*	684
11	*	*	-53	387	-93	*	-73	-167	*	*	173
12	*	*	-60	480	-102	*	-93	-224	*	*	230

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