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REPEAT DETERMINATION OF SH ON TEN FISH MEALS USED IN FIRST SPECIAL PRODUCTS STUDY

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Repeat determination of SH on ten fish meals used in first special products study.

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Summary

Data on the relationship between sulphydryl groups (SH) and mink digestibility obtained in two earlier trials are not readily combinable because different methods and calculations were used in the SH determination. Samples of 10 fish meals which had been kept in the refrigerator for over 3 years were reanalysed for SH. Values were only 61.6% of those determined by the same analyst previously and even lower for two samples which showed a patch of mould growth. As the samples had obviously deteriorated these values were not considered satisfactory to pool with data from the second trial. The original data obtained at Cambridge in Trial 1 was recalculated with correction for blank colour due to the meal samples as now determined to give data for 28 meals all determined in one laboratory and essentially by the same method. The SH content correlated significantly ($P<0.001$) with crude protein digestibility determined in mink according to the regression equation:

Mink digestibity (%) = 3.364SH (mmol/100g meal) + 86.57

However, the use of this equation to predict mink digestibility on future samples is still unsatisfactory as the 95% confidence limits of the predicted mink digestibility are ± 3.9 % units.

Introduction

The determination of the sulphydryl (SH) content of fish meals appeared to be a promising method of predicting mink and salmon digestibility (IFOMA Research Report 1993-1). In this study omitting two low crude protein, high ash meals from a single species origin enabled a significant correlation to be obtained with the remaining eight meals between SH content and digestibility. To confirm and extend this observation a second study was undertaken with a further 18 meals (IFOMA Research Report 1993-5). This confirmed the existence of a significant relationship and again two samples from the same species as before failed to fit the relationship. The quantitative relationship differed from that observed in the first trial. However, the values obtained for SH content in the second series were not directly comparable with the values determined in the first series, primarily due to a necessary modification of the method of analysis to take into account the background colour contributed by the sample irrespective of its SH content. In order to try to pool the data from the two studies the analysis of the 10 meals of the first study was repeated in the same laboratory and using the same method as was used in the second study. In addition the analysis was carried out at two sample weights of 30 and 45 mg crude protein as unpublished observations suggested exceptionally low values were obtained at the low sample weight when the SH content of the sample was low. Subtraction of the blank values becomes more critical when the absorbance reading is low.

Method

SH was determined as detailed in Research Report 1993-5. Determinations were carried out first with a sample size of 30mg crude protein as detailed in Research Report 1993-1 and 8 days later repeated with a sample size of 45 mg crude protein which corresponds to that used in Research Report 1993-5. (NB Research Report 1993-5 erroneously stated the sample size was 50-60 mg; the correct values were 61-78, mean 65.7 SD ±3.5). The samples were also analysed for dry matter by heating for 5h at 70ºC under vacuum and for N by the Kjeldahl method using a Tecator Kjeltec Autoanalyser.

Results

Dry matter and Crude Protein

The samples had been stored in polypropylene pots with metal screw lids in a refrigerator since their previous analysis in 1989. On opening the samples it was immediately obvious that two (9 and 18) were affected with a patch of mould growth. Other samples appeared to be satisfactory. Dry matter and CP determinations were made to check on any changes. For those with mould contamination the sample was taken avoiding the mould areas as far as possible. When the analyses of SH were repeated 8 days later with the larger sample size a further sample (23) was seen to be contaminated with mould. The dry matter of all samples was considerably less than that recorded for the same samples in Report 1993-1, especially for the first two moulded samples (see Table 1). The CP content of the dry matter was 98.0% (SD ± 1.44) of the previously reported value. This small difference could be due to rounding in the published values and to differences due to laboratory methods for N and DM determinations. It was concluded the CP in the DM had not changed appreciably. As the results in Research Report 1993-5 were based on crude protein determinations made in this laboratory, the newly determined values were used in the calculations of SH content in the CP so as not to introduce a new variable (Table 1). The data for SH on a meal basis were adjusted to the original DM contents.

SH determination

The values are given in Table 2 together with the mean values determined in five laboratories as reported in Research Report 1993-1 and the original result of this laboratory corrected for the sample blank now determined.

Values were considerably lower than the previously reported values. In part this was expected as the absorbance readings due to colour from sample blanks and from the reagent were subtracted from the test readings. On average values determined with a sample size of 30mg crude protein did not differ from that determined with 45mg crude protein. However, sample 9 was significantly greater ($P<0.001$) while samples 22 ($P<0.001$) and 23 ($P<0.05$) were significantly lower with the larger sample size or second analysis. Samples 9 and 23, but not 22, were samples observed to be mouldy.

Regression of mink digestibility on SH

The blank corrected values determined at Cambridge for the 10 meals of the first study were added to the reported values in Research Report 1993-5 and the combined data used to examine the regression relationships with SH content of the meal and of the protein. In each

case ash content of the meals was also included as an additional variable. Samples of low crude protein and high ash from a single species source which previously had been identified as outliers were still clearly outliers in the combined data. These four meals were omitted and the analysis repeated.

Mink digestibility was significantly related to SH in the meal by the equation:
Mink dig = 3.364SH (mmol/100g meal) + 86.57
SE of regression coefficient = 0.750; P<0.001;
Residual standard deviation (rsd) = 1.83
Correlation coefficient (r) = 0.48
Percentage variance accounted for = 45.4

The relationship is shown in Figure 1. The four outlying meals that were omitted are also shown.

The corresponding values for SH in the protein were
Mink dig. = 2.167SH (mmol/16gN) + 86.71
SE of regression coefficient = 0.520; P<0.001;
rsd = 1.90; r = 0.44
Percentage variance accounted for = 41.6

The addition of the term ash had no effect on the regression relationships when the four samples of high ash content from one species were omitted.

Discussion

Accuracy of determination

The within laboratory variation (SD on meal basis 0.04-0.05) was a little greater than that previously reported for the same laboratory of 0.035(IFOMA Research Report 1993-5). On this occasion the three replicates for each sample were run in separate batches whereas previously they were analysed simultaneously. The variability is still less than that observed for the pooled within laboratory variance based on the variance of hidden duplicates in the collaborative trial on the same samples (Table 2). The analysis, therefore, can be considered satisfactorily reproducible in the hands of a single operator when samples are analysed closely together in time. Nevertheless some unexpectedly large variations have occurred when the same sample is reanalysed at different times as evidenced by sample 22 in the current study.

Absolute values and importance of blank correction

The mean value for these meals given in Research Report 1993-1 was 1.08 mmol/100g meal. If laboratories weighed out samples of a mean crude protein content of 67.5% to supply 30mg crude protein as directed in the protocol the expected absorbance value can be calculated from:

$$\text{SH (mmol/100g meal)} = \frac{\text{absorbance} \times 40 \times 100}{14323 \times \text{sample wt}}$$

as

$$\text{absorbance} = \frac{1.08 \times 14323 \times 0.0444}{100 \times 40} = 0.1717$$

The mean sample blank absorbance in the current study using 30 mg crude protein samples was 0.0282±0.00894 and the reagent blank was 0.0237±0.00186. Subtracting the sum of these two blanks from the calculated absorbance gives a net absorbance of 0.1198. This is equivalent to an SH content of 0.75, a decrease in value of 0.33 mmol/100 meal as a result of correcting for these two blanks. From Table 2 it is clear that the values differ by more than this amount between the current analyses and the previously reported values, especially for the samples which have become mouldy.

A more direct comparison was carried out by recalculating the values determined on these meals at Cambridge on 18 December 1989 after subtracting the sample blank values now determined. Re-examination of this data revealed that a reagent blank had been subtracted at the time of submission of the data to the collaborative study in 1989. Although not specified in the protocol this was the standard procedure in this laboratory at that time. Consequently, the difference between the adjusted values and the original is not as great as indicated in the above calculation. The new analyses average only 55% (SD 15.7) of the previously determined value in the same laboratory and using the same calculation. Even omitting the mouldy samples 9,18 and 23, the new values are only 61.6% of the corrected earlier values (SD ± 9.22). The conclusion reached is that the SH content of all the meals has deteriorated drastically during storage in a refrigerator during a period of three years and seven months. The increase in moisture content of these meals may have been a contributing factor.

The mean value of SH content of the 10 meals in the first trial, as determined at Cambridge after blank correction, was 1.00 mmol/100g, in the second trial with 18 meals 0.72 mmol/100g. The latter meals were collected over a period of time subsequent to the routine determination of mink digestibility. Consequently, the possibility exists that the SH content of at least some of the meals in the second study had also suffered loss of SH during storage. If so any relationship between SH and mink digestibility may not be correctly reflected in the analyses.

Relationship of SH with mink digestibility

As the repeat determinations on the samples from the first trial indicated serious loss of SH during storage it was not considered appropriate to use these values in combination with those of trial two. Instead the original data determined in 1989 was corrected for the sample blanks as now measured. Thus the SH data of both the first and second trials is essentially determined in the same way and in the same laboratory. As shown in Figures 1 and 2, when the four meals from a single species origin were omitted there was a clear and significant relationship between SH and mink digestibility. However, the range in mink digestibility of interest is relatively narrow and the scatter of SH about the relationship is large. The 95% confidence limits of a predicted mink digestibility for a future sample is approximately ±3.9 % digestibility units. To be 95% certain that the meal has a digestibility greater than 90% requires the SH to be greater than 2.18 mmol/100g meal. Conversely, to be 95% certain a meal is less than 90% digestible requires a SH content of zero. An SH of 1.0 mmol/100g meal corresponds with a mink digestibility of 90% but the 95% confidence limits are 86.1 to 93.9 which virtually covers the whole range. Even the 80% confidence limits are 87.5 to 92.5. If the analysis of SH is done in another laboratory the confidence limits would be greater still.

Another indication of the imprecision of this relationship is the magnitude of the changes in the regression coefficient and intercept values between the data of trial 1 (Research Report 1993-1), of trial 2 (Research Report 1993-5) and now of the combined data.

Expressing the SH as a percentage of the protein did not improve the regression.

Part of the variation in the relationship may be due to the precision of determination of mink digestibility and of SH content. Another part may be due to real changes in SH content between the time of determination of digestibility and of the SH analysis. In future, SH determinations should be made at the same time as the digestibility determination. Both determinations should be made when the meal is fresh. Once residual antioxidants have been used up and the meal starts to oxidise loss of SH groups is likely to be accelerated.

Table 1 Dry matter and crude protein content of samples as reported in 1993-1 and as determined now.

Sample code	Dry matter (%)		Crude protein (% in DM)	
	1993-1	Current	1993-1	Current
9*	92.45	77.62	78.53	76.16
10	92.35	89.13	75.26	73.31
12	88.65	80.12	78.51	75.57
16	89.90	86.79	80.89	78.70
17	91.15	87.13	68.73	67.70
18*	92.15	80.40	65.98	64.67
21	90.60	86.93	75.88	75.66
22	89.05	86.40	74.17	74.09
23♣	88.90	85.66	75.25	73.73
24	89.80	84.02	74.05	73.82

* Sample contained mould

♣ Sample subsequently found to be contaminated with mould.

Table 2 SH content of meal (mmol/100g) and SH content of protein (mmol/16gN) together with SH and mink digestibility previously reported in 1993-1.

Sample	SH in meal				SH in protein		Mink dig
	30mg	45mg	1993-1	1993-1 Cam adj	30mg	45mg	1993-1
9	0.34*	0.54*	1.65	1.46	0.48*	0.77*	92.4
10	0.24	0.21	0.47	0.35	0.35	0.31	87.6
12	0.42	0.38	0.83	0.70	0.62	0.56	88.0
16	0.28	0.26	0.48	0.37	0.40	0.37	86.8
17	0.37	0.35	0.75	0.56	0.60	0.57	82.6
18	0.30*	0.34*	1.46	1.20	0.51*	0.58*	83.8
21	0.51	0.50	0.91	0.74	0.75	0.73	89.5
22	1.40	1.21	2.07	2.23	2.13	1.83	90.8
23	1.06	0.96*	1.99	1.74	1.61	1.46*	91.0
24	0.29	0.29	0.80	0.65	0.44	0.44	89.8
SEM	0.025	0.031	0.093‡	0.075†	0.039	0.048	
Within lab SD	0.044	0.053	0.135†	0.131†	0.067	0.083	
CV	8.4	10.5	12.5	13.1	8.5	10.8	

† Based on the variation between hidden duplicates as the variation between open triplicates tended to be significantly less in a number of cases. SD is for a single analysis to be comparable with current determinations. SEM for Cambridge adjusted values is for a mean of three determinations.

‡ Based on the standard deviation between laboratories. The SEM is for the mean of five laboratories as given in the table.

Figure 1. Relationship between crude protein digestibility in mink (%) and SH content (mmol/100g meal) in 24 fish meals. Four fish meals from a single species source which do not appear to fit the relationship are also shown.

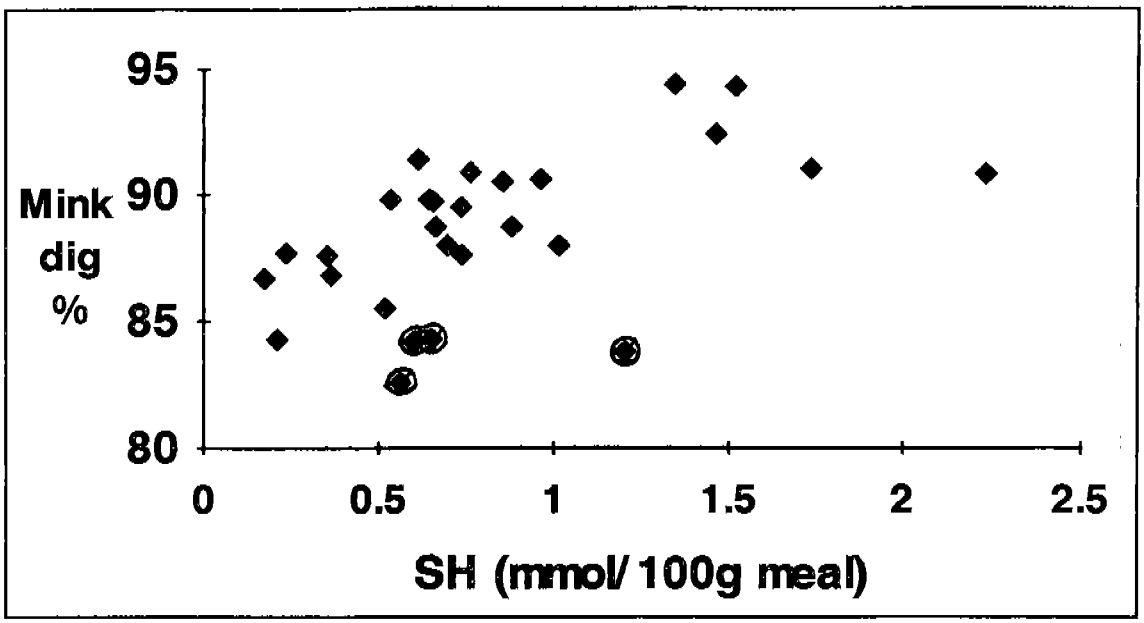

Figure 2 Relationship between crude protein digestibility in mink (%) and SH content of the protein (mmol/16g N) in 24 fish meals. Four fish meals from a single species source which do not appear to fit the relationship are also shown.