ifoma

International Fishmeal and Oil Manufacturers Association

DETERMINATION OF SH CONTENT OF FISH MEALS

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STRICTLY CONFIDENTIAL

DETERMINATION OF SH CONTENT OF FISH MEALS

EXECUTIVE SUMMARY

In Research Report Number 1993-1 it was concluded that none of the chemical tests (pepsin digestibility, pH stat and water binding) were able to predict protein digestibility of fish meals in salmon, mink or rat. However, the SH (sulphydryl) test conducted on eight samples of meal indicated that it might be a reliable predictor of digestibility in target species of animals. Two further meal samples from a single species source did not fit the predicted pattern. Including ash or calcium as further independent parameters in the prediction equation improved the predictive power when all ten samples were taken together but not for the eight selected meals. It was recommended that more work be done on this test.

The present work was undertaken to examine the relationship between SH groups and mink digestibility in a further eighteen samples of fish meal. Again the predictive power of the test looked encouraging, but again two further samples from the same single species source failed to fit the prediction equation. Including ash content in the prediction equation again gave a small improvement in prediction with the full data set of 18 meals but was of no value with the 16 meals remaining after removal of the two divergent samples.

The Scientific Committee recommended that the next step in the assessment of this method would be to collect samples of fish meals which had already been evaluated by salmon digestibility techniques and to determine the SH levels in these meals. In addition, it was noted that the analytical technique had weaknesses in the method and the possibility of improving the technique should be explored.

Determination of SH content of fish meals

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Introduction

In a previous collaborative study (IFOMA 1993) the SH content of fish meals was shown to predict the protein digestibility of 8 of the meals in salmon, mink and rats. However, two meals from a single species source and with a crude protein content of 61-63% did not fit on the regression relationship for the other 8 meals. When all 10 meals were considered together the disparity of the two low protein meals was such that the overall relationship was poor. For example, the SH content accounted for only 24.9% of the variance in salmon digestibility in all 10 meals but 92.1% when the two low protein meals were excluded. Including ash or calcium as further independent parameters in multiple regression equations with the 10 meals increased the response in digestibility per unit increase in SH groups, increased the statistical significance of the SH effect and enabled 82 (calcium) or 86 (ash) % of the variance in digestibility to be accounted for.

The present work was undertaken to examine the relationship between SH groups and digestibility in a further 18 fish meals. True digestibility of the protein in mink was determined at SSF, Norway, but the values were not revealed until after the SH analyses had been performed.

Method

Analysis of SH groups

The method used to determine SH groups was essentially that of Opstvedt et al (1984) as described in Appendix 5 of IFOMA (1993). Detailed differences were:
1. the use of sample weights of between 50 and 60 mg instead of approximately 30 mg crude protein
2. the fish meal stood in 0.2M Tris SDS buffer for 3 hours instead of 2 hours for the protein to dissolve
3. The molar extinction coefficient for 2-nitro-5-thiobenzoic acid used was 14323 as determined in this laboratory
4. a sample blank (same weight of sample carried through the procedure but reacted with solvent instead of DTNB) was determined for each sample
5. a reagent blank (all reagents carried through the procedure but without any sample) was determined with each batch of samples that were analysed
6 the absorbance of the two blanks was summed and subtracted from the test absorbance.

Reagents

(i) 0.2M Na_2EDTA
Dissolve 74.45 g Na_2EDTA in distilled water and make to 1 litre.

(ii) 0.2M Tris buffer pH 8.2,.02M Na_2EDTA, 2% SDS.
For 1 litre solution:
Dissolve 24.228 g tris base [2-amino-2-(hydroxymethyl)-1,3-propanediol] and

20 g sodium dodecyl sulphate in 800ml distilled water in a beaker, add 90 ml 1M HCl and 100 ml of 0.2M Na_2EDTA. Adjust the pH to 8.2 with 1 M HCl, transfer to a volumetric flask and make to 1 litre with distilled water. The buffer was then bubbled with nitrogen for 5 minutes to reduce the amount of dissolved oxygen.

(iii) 0.016M DTNB

Weigh 0.1268g DTNB [5,5'dithiobis(2-nitrobenzoic acid)] Ellman's Reagent, into a 20 ml volumetric flask. Add absolute methanol to dissolve and make to the mark. Pass nitrogen over the surface of the solution to flush the flask before stoppering. Make the reagent up freshly each day. Smaller or larger quantities can be prepared sufficient for one day.

Apparatus

Reactions were carried out in 70 ml Beckman polycarbonate centrifuge tubes, fitted with rubber seal assembly caps. Samples were centrifuged in a Beckman J2-21ME centrifuge. Absorbance was measured on a SP6-500 UV-Visible Spectrophotometer fitted with a 1 cm quartz flow cell (Pye Unicam Ltd, Cambridge).

Procedure

For each meal, four samples of approximately 50mg, milled through 0.5mm screen, were weighed into 4 centrifuge tubes. 8 ml 0.2M Tris buffer pH 8.2,.02M Na_2EDTA, 2% SDS were added and the sample gently dispersed by shaking. Nitrogen was flushed over the solution for a few seconds. The tube was capped with the rubber seal as the nitrogen lead was withdrawn and then sealed with the screw cap. The tubes were allowed to stand for three hours with intermittent (once per hour) gentle shaking. Two centrifuge tubes, each containing 8ml buffer, were similarly set up for reagent blanks (not necessary to leave these for 3 hours).

After 3 hours, to 3 sample tubes and the 2 reagent blanks, 0.5 ml 0.016M DTNB and 31.5 ml methanol were added, mixed, flushed with nitrogen over the top of the solution and capped. To the fourth tube (sample blank), 32 ml methanol were added, mixed, flushed with nitrogen and capped. The solutions were allowed to stand at room temperature for 15 minutes, then were centrifuged at 3000g (6000rpm) for 15 minutes at 20°C. The solutions were filtered through prepared Whatman No 4 paper on filter funnels and the absorbance read as quickly as possible at 412nm against a water blank.

As the centrifuge head took 10 tubes, two samples and two reagent blanks or 2.5 samples were analysed as a single batch. Successive batches were weighed out at intervals in the morning and the assays were completed in sequence in the afternoon.

Crude protein

N was determined by the Kjeldahl method using a Tecator Kjeltec Autoanalyser system. Triplicate 60 mg samples of fish meal were weighed on N free cigarette paper, folded in the paper and placed in 350 ml digestion tubes. 1 Kjeltab CK (3.5g K_2SO_4, 0.4g $CuSO_4.5H_2O$) and 1 Kjeltab TCT (3.5g K_2SO_4, 0.105g $CuSO_4.5H_2O$,0.105g TiO_2) were added followed by 12ml concentrated sulphuric acid. The samples were digested at 440°C on a Tecator Digestion System 20 heating block for 20 minutes after the solution clears (total time about 35 minutes). The tubes were diluted with distilled water, and transferred one at a time to the automatic distillation unit. Excess (25ml) of 40% NaOH were added and the released ammonia distilled into 1% boric acid containing bromcresol green and methyl red mixed indicator. The receiver solution was simultaneously titrated with 0.05M HCl.

Results

Table 1 Crude protein, SH content together with the standard deviation (SD) expressed on a meal basis, SH content of the protein and mink digestibility of fish meals.

Sample code Cambridge	IFOMA	CP %	SH mmol/ 100g meal	SD	SH mmol/ 100g CP	Mink digest ibility %
D1359	SSF9007982	68.47	0.52	0.010	0.76	85.5
D1360	SSF9103255	69.10	0.61	0.055	0.89	91.4
D1361	SSF9103256	67.25	0.66	0.034	0.98	89.7
D1362	SSF9103257	65.75	0.54	0.007	0.82	89.8
D1363	SSF9103257	68.60	0.76	0.048	1.11	90.0
D1364	SSF9201605	72.25	0.23	0.010	0.32	87.7
D1365	SSF9201606	71.95	0.17	0.005	0.24	86.7
D1366	SSF9201607	67.32	0.21	0.025	0.31	84.3
D1367	SSF9202894	71.06	1.34	0.035	1.89	94.4
D1368	SSF9202895	71.52	1.52	0.031	2.13	94.3
D1369	SSF9203806	66.17	0.74	0.020	1.11	87.6
D1370	SSF9203807	66.12	0.88	0.094	1.33	88.7
D1371	SSF9203808	66.01	0.86	0.022	1.30	90.5
D13721	SSF9203809	67.54	0.96	0.042	1.42	90.6
D13722	672	59.11	0.60	0.017	1.02	84.2
D1373	673	60.65	0.65	0.017	1.07	84.3
D1376	14229	68.04	0.66	0.020	0.97	88.7
D1377	14307	67.11	1.01	0.015	1.51	88.0

Discussion

The SH content of the fish meals correlated with mink digestibility of the protein (Figure 1). Mink digestibility was predicted by the equation:
Mink digestibility (%) = 84.23 +6.28SH (mmol/100g meal).
The SH content accounted for 49.2% of the variation in mink digestibity. Each 1.0 unit increase in SH increased mink digestibility by 6.28 percentage units. The standard error of this regression coefficient was 1.50 and the regression was significant at $P<0.001$.

Expressing the SH as % of the protein in the meal did not improve the relationship (Figure 2). The equation became :
Mink digestibility (%) = 84.37 +4.10SH (mmol/100g CP).
The SH content accounted for 41.2% of the variation in mink digestibity. The regression coefficient was reduced to 4.10. The standard error of this regression coefficient was 1.14 and the regression was significant at $P<0.01$.

Two meals of low crude protein content (D13722, D1373) again stood out from the rest. They had low mink digestibility (although one other meal, D1366, had a similarly low digestibility) but only moderately low SH groups. Expressing the results on a protein basis exacerbated the discrepancy of these two meals. The two meals were of the same species origin and were from the same species as meals which gave similar discrepant position in the regressions against digestibility in the first trial (IFOMA, 1993). This suggests that not only the SH content of the protein but another factor such as the collagen content of high ash meals affected digestibility.

Omitting these two meals improved the significance of the regression equations but did not greatly alter the numerical values of the equations. Again expressing the SH on a protein basis did not improve the relationship. The equations were:
Mink digestibility (%) = 85.00 +5.89SH (mmol/100g meal).
S.E. of regression coefficient = 1.20
Percentage variance accounted for = 60.7
Mink digestibility (%) = 84.97 + 4.05SH (mmol/100g CP).
S.E. of regression coefficient = 0.873
Percentage variance accounted for = 57.8

Compared with the above equation on the meal basis with 16 meals (2 omitted) the relationship obtained previously with 8 selected meals differs in the size of the regression coefficient ($P<0.01$).
The previous equation was :
Mink digestibility = 86.65 + 2.46SH
S.E. of the regression coefficient = 0.642
Percentage variance accounted for = 66.3

In the present study SH is a more sensitive indicator of digestibility in that the response in digestibility per unit change in SH is greater. However, some of this advantage is offset by greater variability indicated by the greater SE for the regression coefficient. The ratio of the regression coefficient to its SE is an index of the discriminatory power. This ratio is 4.9 in the present work compared with 3.8 in the previous study.

In the previous study SH varied from 0.47 to 2.07 mmol/100g meal, compared with a range of 0.17 to 1.52 in the present study. Corresponding values for mink digestibility ranged from 86.8 to 92.4 and 84.3 to 94.3. Thus SH content was lower on this occasion than previously. This may be because the assay was done in one laboratory only this time whereas previously the values used were the mean of five laboratories. In addition, the method used in the present study differs in that sample blanks were subtracted from absorbance units before calculating SH content. Typically this amounted to 0.03 to 0.04 absorbance units, accounting for 20 to 25% of the test value at low SH content but less than 10% at high SH content. This blank correction could account for the greater regression coefficient in the present work but the extent of the difference in the two studies between SH content of the best meals with similar mink digestibility still suggests an undesirable variation in the analysis.

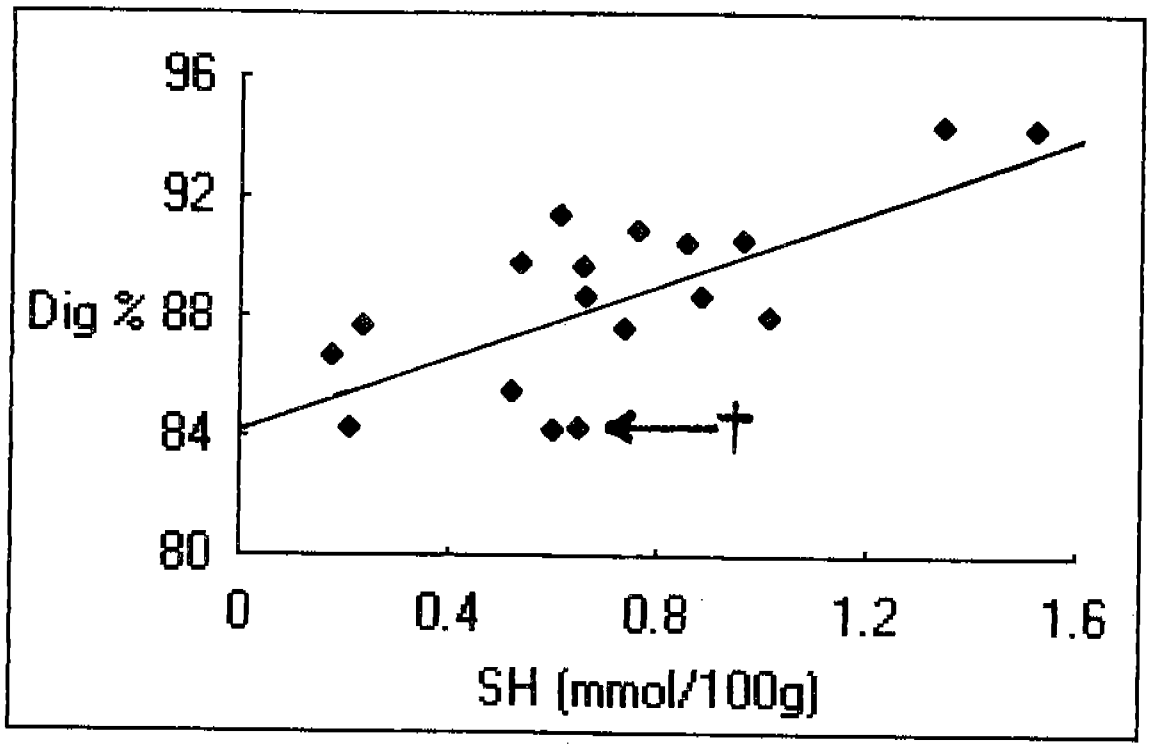
The reproducibility of the analysis in the hands of a single analyst is indicated by the standard deviations given in Table 1. The pooled mean Standard Deviation was 0.035 or 4.9% of the overall mean of 0.718 mmol/100g meal. This is similar to the Within laboratory coefficient of variation (C.V.) of 7.2% obtained in the collaborative trial (IFOMA, 1993). However, these replicates were run side by side. Greater variation may be expected if replicates are run at different times or on different days. Even greater variation was observed previously between laboratories where the C.V. was 19.3% (IFOMA, 1993).

References

IFOMA (1993) Research Report Number 1993-1. Tests to predict the quality of fish meals for special uses.

Opstvedt J., Miller, R., Hardy, R.W. and Spinelli, J. (1984). Heat-induced changes in sulfhydryl and disulfide bonds in fish protein and their effects on protein and amino acid digestibility in Rainbow trout (Salmo Gairdneri). J. Food Agric. Chem., 32: 929-935.

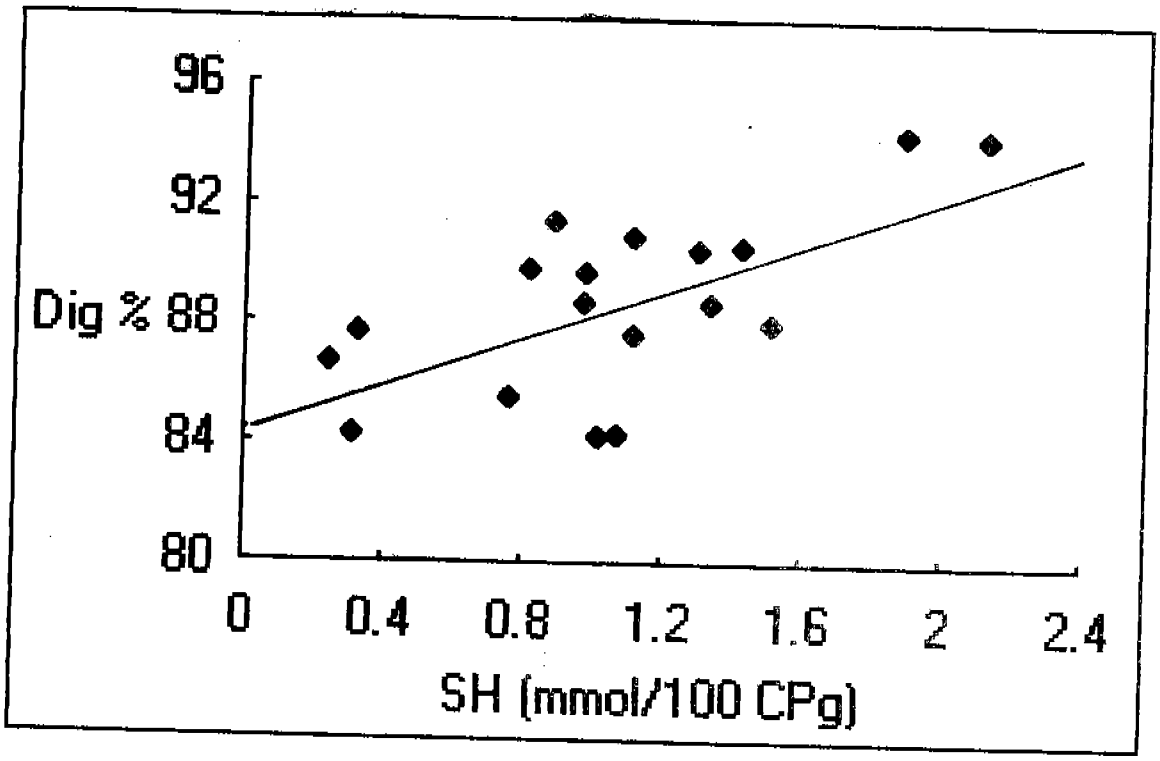
FIGURE 1
MINK DIGESTIBILITY AND SH CONTENT (MMOLES/100G MEAL) OF FISH MEAL



Mink digestibility (%) = 84.23 + 6.28SH (mmoles/100g)
Residual standard deviation = 2.193
Percentage variance accounted for = 49.2

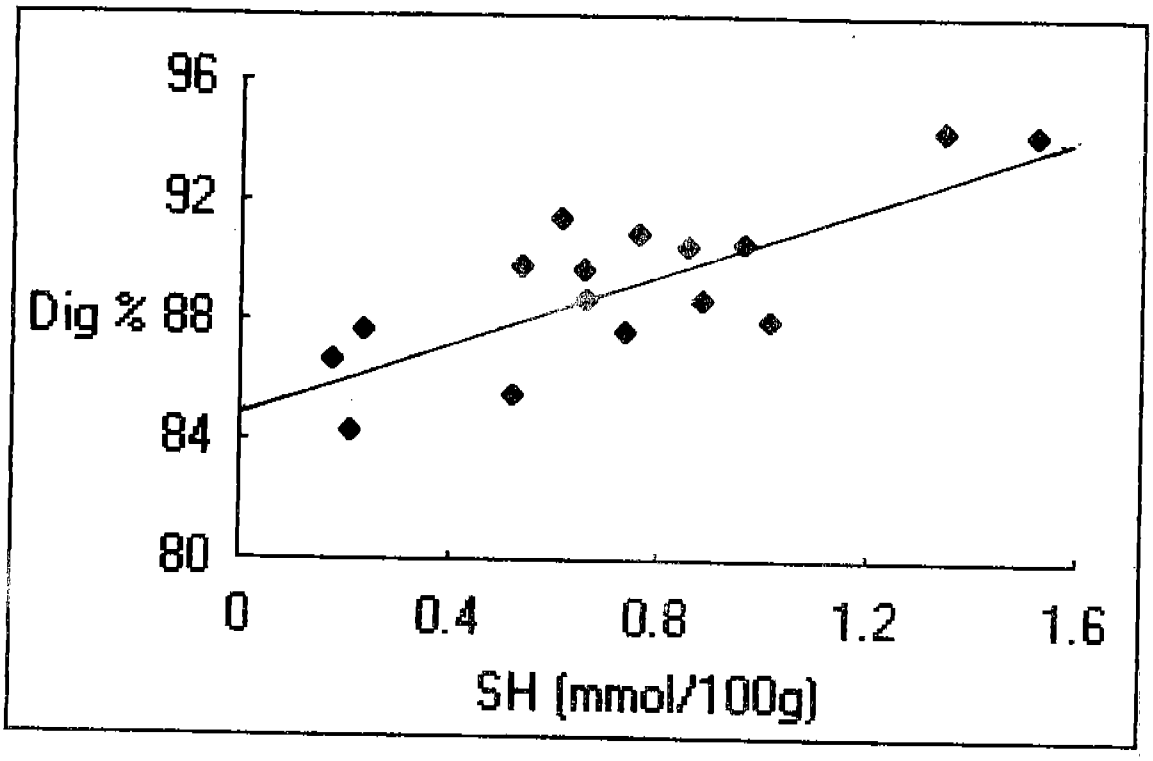
† samples D13722 and D1373

FIGURE 2
MINK DIGESTIBILITY AND SH CONTENT OF PROTEIN (MMOLES/100G CP) OF FISH MEAL



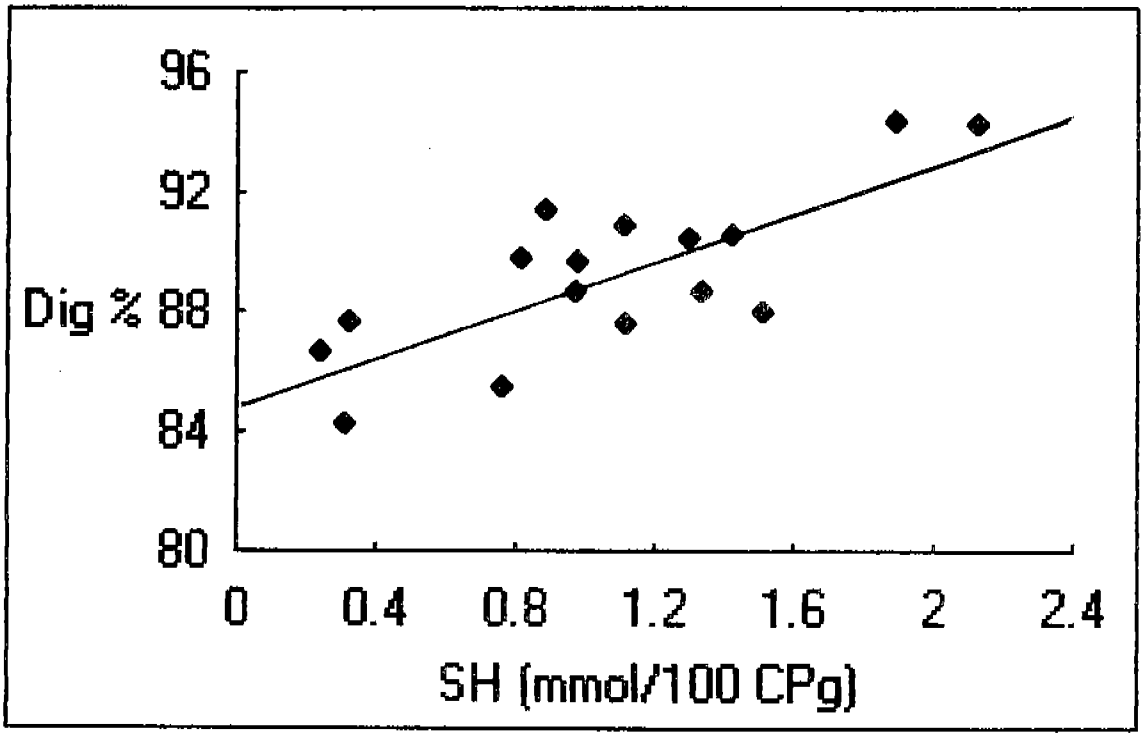
Mink digestibility (%) = 84.37 + 4.10SH (mmoles/100g CP)
Residual standard deviation = 2.359
Percentage variance accounted for = 41.2

FIGURE 3
MINK DIGESTIBILITY AND SH CONTENT (MMOLES/100G MEAL) OF FISH MEAL AFTER OMITTING TWO LOW PROTEIN MEALS



Mink digestibility (%) = 85.00 + 5.89SH (mmoles/100g meal)
Residual standard deviation = 1.740
Percentage variance accounted for = 60.7

FIGURE 4
MINK DIGESTIBILITY AND SH CONTENT OF PROTEIN (MMOLES/100G CP) OF FISH MEAL AFTER OMITTING TWO LOW PROTEIN MEALS



Mink digestibility (%) = 84.97 + 4.05SH (mmoles/100g CP)
Residual standard deviation = 2.359
Percentage variance accounted for = 57.8

Addendum

An examination of the use of ash content of fish meals as a covariate with SH content to predict mink digestibility.

In the previous collaborative study (IFOMA 1993) two meals of high ash content did not fit to the general relationship between SH content of fish meal and mink digestibility observed with the remaining meals. Including ash content of the meal as a second variate greatly improved the prediction of mink digestibility when all 10 meals were considered but had no improving effect with the 8 meals remaining after omitting the two abberrant meals.

In the current study a similar situation arose in which two meals from the same species source as in the previous trial appeared to be outside the general relationship described by the remaining 16 meals. Consequently, ash content was determined by heating for 6h at 580 °C. The relationship between SH in the meal or SH in the protein was examined with ash content as an additional variable for the complete data set of 18 meals and also after omitting the two meals that appeared to deviate.

Results

Table 2 Crude protein and ash content of fish meals.

Sample code Cambridge	IFOMA	CP %	Ash %
D1359	SSF9007982	68.47	10.76
D1360	SSF9103255	69.10	14.14
D1361	SSF9103256	67.25	14.95
D1362	SSF9103257	65.75	14.02
D1363	SSF9103257	68.60	15.81
D1364	SSF9201605	72.25	11.14
D1365	SSF9201606	71.95	8.93
D1366	SSF9201607	67.32	13.65
D1367	SSF9202894	71.06	10.23
D1368	SSF9202895	71.52	9.58
D1369	SSF9203806	66.17	14.26
D1370	SSF9203807	66.12	13.67
D1371	SSF9203808	66.01	14.82
D13721	SSF9203809	67.54	13.68
D13722	672	59.11	17.70
D1373	673	60.65	17.68
D1376	14229	68.04	15.06
D1377	14307	67.11	15.13

Regression equations without and then including ASH as a variable are shown in Table 3.

Including ASH increased the variance in mink digestibility accounted for by SH content of the meal from 49.2 to 56.2%. ASH content was negatively related to mink digestibility but the relationship did not achieve statistical significance at the 5% level. When SH was expressed in terms of content of the protein similar relationships were observed and this time the negative effect of ASH was statistically significant.

When the two meals were omitted SH alone accounted for more of the variance in mink digestibility than using ASH as an additional variate with all 18 meals. Inclusion of ASH in the equations with 16 meals did not significantly

improve the prediction with either SH in the meal or SH in the protein. Indeed the variance accounted for actually decreased.

Table 3
(a) Regression equations relating to all 18 meals

Mink digestibility % = 84.23 + 6.28 SH
Standard error of regression coefficient 1.50 t =4.18 P<0.001
Percentage variance accounted for 49.2

Mink dig % = 89.39 + 6.04 SH - 0.366 ASH
SE of regression coefficients SH 1.40 t = 4.31 P<0.001;
ASH 0.195 t = 1.88 P>0.05
Percentage variance accounted for 56.2

Mink digestibility % = 84.37 + 4.10 SH in CP
Standard error of regression coefficient 1.14 t = 3.60 P<0.01
Percentage variance accounted for 41.2

Mink dig % = 90.36 + 4.10 SH in CP - 0.439 ASH
SE of regression coefficients SH in CP 1.03 t = 3.99 P<0.01;
ASH 0.202 t = 2.17 P<0.05
Percentage variance accounted for 52.3

(b) Regression equations relating to 16 meals (omitting 2 meals)

Mink digestibility % = 85.00 + 5.89 SH
Standard error of regression coefficient 1.20 t =4.92 P<0.001
Percentage variance accounted for 60.7

Mink dig % = 85.85 + 5.55 SH - 0.064 ASH
SE of regression coefficients SH 1.24 t = 4.74 P<0.001;
ASH 0.210 t = 0.30 Not Significant
Percentage variance accounted for 58.0

Mink digestibility % = 84.97 + 4.05 SH in CP
Standard error of regression coefficient 0.873 t = 4.64 P<0.001
Percentage variance accounted for 57.8

Mink dig % = 86.46 + 4.06 SH in CP - 0.113 ASH
SE of regression coefficients SH in CP 0.896 t = 4.53 P<0.001;
ASH 0.216 t = 0.52 Not Significant
Percentage variance accounted for 55.5

Discussion

The results are essentially similar to those obtained in the previous collaborative trial (IFOMA 1993). Inclusion of an ASH term improves the prediction of mink digestibility from SH, measured either in the meal or in the protein, when all meals are considered. However, including ASH has no benefit when 2 meals from a single species source are omitted.

The effect of including ASH was less marked in the 18 meals of this trial compared with the effect in the 10 meals of the previous trial. In part this reflects the smaller effect of 2 divergent meals in a greater population. In addition these two meals had a lower ash content (17.7%, 17.7%) than previously (19.0%, 21.1%). The remaining meals had the same mean ash content but covered a wider range in the current data set (mean 13.1 range 8.9-15.8

in current set compared with mean 13.1 range 10.7-14.8 in the previous set). The lack of any significant effect of ASH in the prediction equation for mink digestibility with the restricted set of 16 meals, despite the wide range in ash content, suggests that the problem of the two divergent meals does not lie in a greater content of bone protein per se but that there is some factor other than SH which causes a reduced protein digestibility and it is coincidental that these meals are low in protein and high in ash.

In the light of one fish species difference in the relationship between SH and mink digestibility, the relationship should be examined within fish species and a pooled relationship only used where it is shown that the same relationship holds for each species.