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SPECIAL PRODUCT FISH MEALS - AN OUTLINE OF PROJECTS UNDERTAKEN BY ASSOCIATION MEMBERS

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SUMMARY AND CONCLUSIONS

A brief account of the scope of the work undertaken by Members in connection with the development of special product fish meals is given. Results of the work are indicated as far as possible whilst respecting requests from Members to treat certain aspects of the work as confidential.

Freshness of raw material and processing temperature exposure may be two important factors influencing nutritional value of the products. However, in much of the work undertaken, because both varied, and also other factors may have changed, e.g. the plant used, the amount of information specifically relating to either freshness or processing temperature is limited.

Freshness and Species of Raw Material

Freshness of raw material would appear to be important in fish meals for early weaned pigs, mink and trout but there is insufficient evidence available to judge its importance for salmon. There is an urgent need to obtain information in this connection, using salmon of different age. It would appear that broiler chickens are not sensitive to freshness of raw material.

Freshness has been described in relation to the TVN content of raw material at the time of processing. There appears to be no comparison of meals from different fish processed at the same TVN, yet it is known that similar TVN levels can be reached in different types of fish after different storage periods at similar temperatures following catching.

Processing Temperature Exposure

Processing temperature exposure appears to be important in the production of fish meal for salmon. There are no data with early weaned pigs with regard to fish meal processing temperature. There appears to be no growth response of trout to meals processed at different temperatures is - in several Danish trials and some comparisons in a Norwegian trial there was no response, but in one comparison in the Norwegian work there was a response (see page 6). All the trials were done with freshwater rainbow trout. There is a need for trials with rapidly growing sea trout as there is some suggestion from a fish feed company that they respond to low temperature processed fish meals.

With mink a Danish trial showed only a small growth response, whereas in two Norwegian trials there were responses in growth with reduced processing temperature and also with commercial low temperature meals e.g. Norse LT94, and digestibility was increased.

Analytical Methods to Measure Quality

A variety of chemical methods have been developed for estimating the quality of Special Product Fish Meals. The use of these methods can be summarized as follows:

Crude protein: required for statutory purposes, but does not change appreciably with either freshness of raw material or even extreme overheating.

Amino acid content: direct determination of amino acid content of an individual sample is expensive and time consuming and significant variation occurs between laboratories measuring amino acids in the same sample which further reduces the value of amino acid analysis as a routine procedure for use in sales promotion. Serious overheating of meal or use of stale raw material can decrease the content of more sensitive amino acids in the meal.

Water soluble crude protein: reflects the amount of solubles added to presscake which may be a reflection of freshness of raw material.

Titration to pH10: appears to be a reasonable indicator of content of solubles and therefore, indirectly freshness of raw material. The results are confused if formaldehyde is used during processing. The method is simple and quick and merits further investigation.

Biogenic amines: determined as a proportion of water soluble protein may indicate age or quality of the solubles. Addition of formaldehyde to a meal may render analysis of the amines impossible.

TVN in the meal: has little value in predicting either freshness of raw fish or nutrient/toxic components in the meal.

Dilute pepsin digestibility (Torry method): is highly correlated with mink true digestibility and processing temperature exposure. However both mink digestibility and pepsin solubility are rather insensitive tests of the improvement in nutritive value and animal growth that appears to be achieved with low temperature processed meals.

Multi-enzyme digestibility and pH stat method: can be used for assessing processing temperature exposure. The pH stat method is quick (10 minutes) and correlates with rat digestibility. Meals prepared from stale material gave overestimates of rat true digestibility by the pH stat method. Similarly the value of such meals for growth of early weaned pigs, mink and trout was overestimated. As with pepsin solubility, the test appears to work best when raw material is known to be fresh.

Dye binding capacity: is likely to distinguish the very bad sample from the very good, but appears to be inappropriate for distinguishing between low temperature meals and normal meals.

Sulphydryl and disulphide bonds: appears to be a sensitive indicator of processing temperature and significantly correlates with mink true digestibility and salmon growth. Further work is required to test this method with commercial samples and to correlate with animal performance in salmon, trout, mink and early weaned pigs at various stages of growth.

INTRODUCTION

At the meeting of the Association's Executive Council in Dubrovnik, September 1987, it was proposed that before drafting detailed research proposals for special product fish meals, all Members should provide the Association with information available to them, including data on freshness of raw material, processing conditions and quality control criteria to produce special product fish meals. Members were invited to submit all relevant information to headquarters (see letter of 23rd November 1987 ref. IHP/CM/1988/7). It was stressed that this information would be treated in confidence.

From the information submitted, an outline of the scope of the work done on special product fish meals has been drawn up and is given below. Whilst full details have not been given, in order to respect confidentiality, sufficient detail is provided to show that the objectives of the Association's Special Product Project do not duplicate those of work already undertaken by Members.
Sources of information which has been published, or is in press, are given.

Because freshness of raw material and processing temperature exposure may be important factors influencing the nutritional value of special product fish meals for the purpose intended, work already undertaken by Members which provides information in this regard will be given prominence. In many trials, both freshness of raw material and processing temperature varied, or were not controlled, and sometimes other factors varied too, such as type of fish, processing plant, etc. Such trials do not allow the separate effects of freshness or processing temperature to be assessed.

Full reports were available to an IAFMM 'in house' working group (Drs. Miller, Duthie, Barlow and Pike) who have now produced a protocol for a Special Products Project to be undertaken by the IAFMM.

BIOLOGICAL ASSAY

1. FRESHNESS OF RAW MATERIAL

1.1 Early Weaned Pigs

Only one experiment investigating fish meal for early weaned pigs has been reported to the Association. This trial, organised by the Danish Association is reported on page 12 of the Association's Technical Bulletin Number 17. Comparing whole fish meals made from different raw materials of different freshness (TVN max. 70 and max. 120/140), piglets from 4 to 10 weeks of age grew 7% faster and had better feed conversion (5% improvement) when fed fish meal from fresh raw material. This comparison could have been affected by different species of fish or processing conditions as the fish meals were produced by different factories.

1.2 Fish

In a trial designed primarily to investigate different methods of processing fish meal, the Andelssild factory in Denmark included fresh raw material (sandeel 2 days after catch, TVN 30mgN/100g) and stale raw material (sandeel 9 days after catch, TVN 130mgN/100g). When fed to trout weighing 50g, in water at 12°C, growth was improved by approximately 5% with fish meals made from the fresher fish.(1)

1.3 Mink

Fish meals made from fresh mackerel (TVN 22mgN per 100g fish) or stale mackerel (TVN 100mg N per 100g fish) were fed to mink kittens. The content of biogenic amines (cadaverine, histamine, putrescine and spermidine) in the meals averaged 450mg/kg for the fish meal from fresh fish and 9160 mg/kg for the fish meal from stale fish. In the first four weeks of the growth trial growth of the mink kittens on the stale material was 17% below that of those on the fresh material. Feeding the stale material was subsequently abandoned because of illness and mortality in the mink kittens, believed to be due to the high amine content of the diet, especially histamine. There was an interaction between the effects of freshness and drying temperature on digestibility in adult mink. Drying at 60°C, digestibility was 2% to 3% units lower for the stale material compared with the fresh; drying at 140°C digestibility of the stale and fresh materials were similar.

2. PROCESSING TEMPERATURE EXPOSURE

2.1 Early Weaned Pigs

No trial results are currently available with early weaned pigs comparing fish meals made under conditions where processing temperature exposure was the only variable.

2.2 Fish

The Danish equipment manufacturer Atlas reported a trial (2) with trout fed diets (six dietary treatments) based on fish meals produced using different types of drying including normal, freeze dried, vacuum dried and hot air dried. Methods and drying conditions, etc., are shown in appendix table 1. Comparisons were made of steam drying (steam temperature 160°C), hot-air drying (inlet air temperature 250°C, 550°C or 600°C), vacuum drying (steam temperature 120°C) and freeze drying, using the same raw material with TVN of 80 to 90 mg N per 100g. A growth trial with trout fed diets based on the fish meals was undertaken by the Danish Trout Culture Research Station in Bronx. Differences in growth of fish were not significant and it was concluded that with fresh raw material the methods of drying did not affect trout growth. Surprisingly the slowest growth was with the freeze dried whole meal. Growth was variable ranging from 1.19% to 1.64% of body weight per day (water temperature 12°C; fish liveweight at start 50g).

The trial reported earlier (see 1.2) also compared fish processed by indirect steam drying (steam termperature 120°C), freeze drying or roller drying (160°C drum temperature). The trout received three different levels of protein and oil in the diet. Growth in the three trials ranged from 0.95% to 1.46% per day, but there were no differences due to processing method. Increasing the amount of protein and oil fed resulted in increased growth, indicating that fish could respond to dietary treatment.

In a Norwegian experiment capelin was cooked at 98°C and dried at temperatures of 60, 70, 80, 90 and 110°C. The fish meal produced was fed to salmon weighing approximately 345g. Growth decreased progressively with increasing cooker temperature from 70°C:

Temperature of drier (°C)	60°	70°	80°	90°	110°	SEM
Growth (g)	289	290	278	260	217	12.7

SEM = Standard error of treatment mean.

Growth with meal dried at 110°C was significantly less than that with meals dried at 60°, 70° or 80°C.

The same meals from the Norwegian experiment above were fed to mink and showed decreasing digestibility:

mink digestibility	95.3	94.5	94.3	93.7	92.7

This trial will be reported in the Association's forthcoming Technical Bulletin on Feeding of Rainbow Trout and Atlantic Salmon (3).

2.3 Mink

The fish meals prepared by three different drying procedures, (steam drying - A, hot air drying (250 °C) - B and vacuum drying), reported by the Danish company Atlas (see section 2.2) were also fed to mink in a growth and digestibility trial undertaken by the Institute of Animal Science in Hillerod, Denmark (2). There was no difference in digestibility of dietary protein, carbohydrate or fat for the three fish meals. Although growth differences did not achieve statistical significance, those mink receiving the vacuum-dried whole meal grew approximately 10% faster: A- 215 64g, B- 215 67g, C- 237 70g. The high standard errors indicate that there was large between animal variability in growth.

In contrast to the Danish trial, two growth and several digestibility trials with mink in Norway have shown that both growth and digestibility in mink improved when the fish meal they received was processed at a lower temperature. For example, fish meals produced by drying at temperatures ranging from 60°C to 110°C fed to mink resulted in progressively decreasing digestibility with drying temperature increase:

dryer temperature °C	60	70	80	90	110
true protein digestibility	95.3	94.5	94.3	93.7	93.2
SE	0.6	0.7	0.4	0.5	0.7

From this and other trials it was found that when meal processing temperature exceeded 70°C to 80°C protein digestibility in mink decreased progressively with increasing temperature up to 140°C. Protein digestibility of meal processed at 140°C was 3.3% units lower than that for meal processed at 60°C. These results are based on a total of 195 observations.

Norwegian trials have also investigated combined effects of dryer temperature and drying period (temperature exposure) and also effects of different drying temperatures at different stages of fish meal processing - in the cooker, evaporator and dryer. Extensive laboratory analysis and mink digestibility have been carried out on the fish meals produced.

3. TRIALS IN WHICH BOTH FRESHNESS OF RAW MATERIAL AND PROCESSING TEMPERATURE VARIED - COMMERCIAL FISH MEALS

Extensive data with commercial fish meals produced from raw material of different freshness and different processing temperature exposure have been made available to the Association. Details of the type of raw material and

factory processing equipment used are generally not available. An outline of this data is presented below, along with any indications it might give about the effects of raw material and/or processing.

A large survey undertaken in Denmark involving 134 samples from 10 factories, gives details of the raw material, TVN value, use of antioxidant and any preservative, and in some cases indicates meals which appeared dark through overheating. All the meals were analysed (moisure, protein, oil, amino acids, pH stat, titre value) and some were subjected to growth trials with chicks, mink, trout and piglets. Rumen degradation of some of the meals was determined using cattle. The information is reported in the Danish Association's book 'Quality Criteria for Fish Meal', March 1983. It has been circulated on a confidential basis to some feed companies. Because its main objective was to evaluate meals which could be produced on a commercial scale in Danish Factories, it has not separated out individual factors such as raw material freshness, with the exception of one trial, reported earlier in section 1.1. It attempts to relate laboratory assessment of fish meals to bio-assay - growth and digestibility data.

Some of the analyses on raw material (TVN), fish meal (pH-stat and titration value) and chick and rat bioassay data are shown in appendix table 2. The biological values of the fish meals determined in rats appear to be lower for those fish meals made with raw material with high TVN (120, 140 and 250mg per 100g fish) though other factors would have affected biological value. Chick growth and feed conversion, on the other hand, do not appear to be affected by TVN value in the raw material with the possible exception of some fish with very high TVN (250 mgN per 100g fish). Chemical analyses of raw material and fish meals and growth rates for trout are shown in appendix table 3. These fish meals are mainly different meals to those in the former tables. The four best meals in terms of trout growth were produced from fish with low TVN values (under 50 mg per 100g fish). The two meals made from fish with highest TVN gave low growth rates. The pH stat results did not appear to correlate with trout growth.

In Norway an extensive series of trials were undertaken with the special product meals Norseamink[1], and Norse LT94[2]. These trials have been undertaken by the Herring Meal and Oil Research Institute (SSF) with early weaned pigs, salmon, trout and mink. The former meal must be made from fish with a TVN value of not more than 90, the latter not more than 50mgN per 100g fish. Detailed specifications are given in Appendix Table 4. The Norse LT94 is produced using low processing temperatures involving some types of dryers which have not previously been commonly used in commercial fish meal production. The temperature in the dryer is not specified, but the factory must have a process which under commercial running conditions can produce meal that gives a digestibility value of at least 90% in mink. The meal must also support growth of mink similar to that of a reference standard Norse LT94. Having met these conditions, the factory may then apply for a licence to produce Norse LT94. For day to day factory control, a dilute pepsin solubility test is used, a value of at least 94 having been found to correspond to a mink digestibility of 90% or over.

1. Made from fresh fish, TVN 90mg per 100g fish.
2. Made from fresh fish, TVN 50mg per 50g fish and processed at low temperature, giving minimum pepsin digestibility of 94% and in vivo mink digestibility of 90%.

Norwegian tests on fish meal from indirect steam dryers have shown mink digestibility values which would correspond to a drying temperature of around 140 degrees C, based on values obtained previously drying meals over a range of temperatures (4).

It is claimed that mink digestibility data correlates well with salmon growth. The two do appear to be related in the limited data received by the Association, but the degree of correlation (correlation coefficients) has not been established.

The Norwegian data shows that on average mink digestibility is about 5% units higher for Norse LT 94 than for Norseamink, based on assessment of approximately 150 samples over the past five years subjected to mink digestibility trials.

In four trials with salmon parr (approximately 100g liveweight) there were seven comparisons of Norse LT 94 v Norseamink. In six of these there was a positive response in growth to the LT meal ranging from 5% to 30%. There was no response in the seventh comparison using diets with a high protein content. In a further trial with salmon of initial weight 500g, a growth response of around 15% was obtained using LT in comparison with Norseamink fish meals. Furthermore the difference in growth rate was maintained up to 2kg liveweight when the experiment was terminated. This trial is described in the Proceedings of the Association's 26th Annual Conference, 1986. The overall average response in growth of salmon to the LT meal in these trials was in the range 10% to 15%. This would represent a major increased financial return to the salmon farmer as he currently receives £3,500 to £4,000 per tonne salmon produced.

In a trial with trout of approximately 170g liveweight held in tanks at different water temperature (7°, 11° and 14°), there was no response to LT meal at the lower temperatures but growth was around 10% better at the higher temperature with the LT meal, compared with Norseamink (217v197g over a nine week period). These growth rates are very low due, it is suggested, to a high stocking rate in the tanks and a low protein content in the diet (protein 29% of the metabolisable energy).

The trials comparing fish meal produced from very fresh fish (max. TVN 50mg N per 100g fish) processed at low temperatures with fresh fish (max. TVN 90mg N per 100g fish) processed at normal temperatures show marked improvement in growth of salmon and mink. The response to LT meals compared with regular meals where freshness of fish is not controlled may show an even bigger improvement in growth, though such comparisons are not available at the present time.

In two trials with mink kittens growth was improved by 19% and 6% comparing LT with Norseamink meals in their diet.

In a trial with piglets weaned at three weeks of age, on trial until they reached 22kg liveweight, growth with the LT meal was around 23% higher than with the Norseamink.

SIGNIFICANCE OF ANALYTICAL METHODS IN THE ESTABLISHMENT OF SPECIAL QUALITY MEALS.

The general purpose of analytical data are:

1. To meet statutory requirements
2. To indicate the nutritive value of the sample of fish meal and thereby assist sales promotion
3. To monitor or control processing.

In the present context, only 2 and 3 above are considered further.

The analytical methods chosen may directly measure the required parameter or may determine a related parameter which is easier, quicker or cheaper to measure. The latter will be particularly relevant to the monitoring of processing. Direct determination of nutrient content, nutrient availability or digestibility and of known toxic or anti-nutritional factors is desirable to indicate nutritive value. Establishment of optimum or desirable levels of nutrients, toxic and anti-nutritional factors requires the detailed study of the relationships between the measured chemical entities or *in vitro* laboratory simulation tests and animal performance.

The preceding review of data submitted to IAFMM indicate the possible value of using fresh raw material and of processing this at lower temperatures than are used in conventional fish meal production to produce special products for certain species or stages of growth of livestock. While it is clearly essential to develop methods to monitor and control freshness of raw material and processing temperature, attempts to measure these parameters in the finished product are at best likely to yield only indirect indications of nutritive value. For example, temperature of processing might be monitored by following the fate of a heat sensitive but otherwise inert additive to the raw material. For nutritional purposes, analyses showing improved nutrient content or availability are necessary. Manufacturers should regard any attempt to provide data substantiating freshness or processing *per se*, indicating an improved value through special processing, as an interim measure only. Current and some potential methods are reviewed against this background.

4. CRUDE PROTEIN

Required for statutory purposes, but does not indicate amino acid content or the availability of critical essential amino acids, does not change appreciably with either freshness of raw material or even extreme overheating.

5. AMINO ACID CONTENT, PARTICULARLY LYSINE, METHIONINE, CYSTINE, THREONINE AND TRYPTOPHAN.

The amino acid composition of the protein of specific tissues, e.g. muscle, bone, liver, is reasonably constant but variation in the proportion of tissues e.g. whole fish, offal, solubles added to press cake, will affect amino acid content of fish meal. In the Danish study of 134 samples of commercial Danish meals, (Lewy, 1983) the 95% confidence intervals for the content (g/kg meal) of lysine, threonine, and methionine plus cystine of any one meal were 10.8, 12.5 and 14.5% of the mean values respectively. Such variation is nutritionally very significant. The variation between the meals was reduced when amino acid

content was expressed as % of the protein (g/16gN). The corresponding confidence limits were 7.9, 8.7 and 11.1% of the mean values. Thus, a better prediction of the amino acid content of an individual meal can be made from a known crude protein content coupled with average values for the amino acid content of fish meal protein. Furthermore, significant differences occurred between individual factories in the lysine content of their meals. The report does not clarify whether this was due to differences in crude protein content or to differences in the amino acid content of the protein. Use of determined crude protein coupled with a factory specific mean amino acid analysis might give improved prediction. Nevertheless nutritionally important variation is still unaccounted.

Direct determination of amino acid content of an individual sample is still an expensive and time consuming analysis, although development of HPLC techniques is likely to assist in this direction. In the Danish study all analyses were carried out by a single laboratory. The IAFMM collaborative study of ion-exchange and GLC amino acid methods (Miller et al., in press) indicated significant between laboratory variability which further reduces the value of amino acid analysis as a routine procedure for use in sales promotion.

The Danish study also established a significant relationship between Dye Binding Capacity and content of lysine, threonine, methionine and methionine plus cystine. Although DBC measures reactive-lysine plus histidine and arginine, the relationship with threonine, methionine and cystine reflects the constancy of fish protein. However, the accuracy of the relationships is such that individual amino acids are estimated to about +/- 10% of the real value. Consequently, the use of DBC is likely to distinguish the very bad sample from the very good but an average value could be either good or bad.

As raw material deteriorates the protein is hydrolysed, and the water soluble crude protein increases. Consequently, return of the solubles in whole meal results in higher amino acid content than in press cake meal. With further deterioration, amino acids are decarboxylated to give amines, and deaminated yielding ammonia by bacteria with consequent loss of amino acids. In the Danish trials two experimental meals made from high TVN raw material had the lowest content (g/16gN) of histidine (1.84, 1.86 v 1.90-1.98), lysine (7.25,7.47 v 74-8.19), arginine (5.13,5.43 v 6.12-6.27), tyrosine (22.77,3.01 v 3.21-3.61) and of other amino acids of the whole meals. Use of fresh raw material can be advocated simply from the reduction in solubles that need to be added back and the increase in amino acid content. In addition, there is the advantage of minimising the production of nutritionally useless ammonia and amines which also may have adverse physiological effects.

The Danish data also clearly shows the adverse effect of serious overheating during drying on the amino acid content, particularly of the more heat sensitive and nutritionally important amino acids. A presscake meal dried excessively had only 0.5% less crude protein but 51% less cystine, 29% less threonine, 25% less lysine and 17% less methionine than a meal prepared from the same raw material but dried without causing browning. Other studies have shown destruction of amino acids is not appreciable until temperatures are in excess of 115 C. Amino acid composition is not expected to differ between low temperature-dried and normally-dried meals.

Summation of the nitrogen contributed by all the amino acids, including an allowance for tryptophan which was not measured in the Danish study, accounts for only 78% of the total N of average fish meals. Ammonia-N can be determined simultaneously from the chromatogram. Part of this will be amide-N released from the protein during hydrolysis and part from the destruction of

tryptophan. Therefore total N - amino acid N is only an approximate estimate of non-protein N and also suffers from the accumulation of errors of estimation of each amino acid.

6. WATER SOLUBLE CRUDE PROTEIN

The water soluble protein content of a meal reflects the amount of solubles added to presscake. This may or may not be a reflection of the freshness of raw material and the extent of hydrolysis of the protein.

7. TITRATION TO PH 10.

As protein hydrolyses during storage of raw fish and releases free amino acids the amount of alkali needed to titrate the resultant meal to pH 10 increases. Hydrolysis of oil in the raw material increases the free fatty acid content and also increases the titre. Addition of formaldehyde, by reacting with amino groups, reduces the alkalinity of the meal and consequently also increases the titre. The titration value is, therefore, affected by a number of independant as well as correlated factors. In a study reported from Andelssild, the pH 10 titre correlated with water soluble protein with an R^2 of 91.4%.

In the Danish study, Jorgensen et al observed a correlation coefficient of 0.7 between titre to pH 10 and growth of mink, with the better quality meals averaging a titre of 64 ml 0.1N NaOH/10gDM and poorer meals averaging 96ml. Although a poor meal made from material with a TVN >250 mg N/100g had a high titre, so did a formalin-treated meal made from low TVN raw material. Therefore the correlation cannot be taken as evidence of the importance of freshness of raw material for mink. Nevertheless, providing formaldehyde has not been added, the titre value does appear to be indicative of content of solubles and, therefore, indirectly of freshness of raw material. The method is simple and quick and merits further investigation.

8. BIOGENIC AMINES

These are produced by bacterial decarboxylation of amino acids. They can be separated and individually determined by HPLC. At high concentrations they are known to have adverse physiological or pharmacological effects. Critical concentrations at which these effects become manifest are not known. Further work to establish such limits are urgently required. Meanwhile it will be prudent to keep the content of biogenic amines as low as possible. Studies by Andelssild have indicated that water soluble protein, or titre to pH 10 as an indirect estimate, indicates the amount of solubles added but not necessarily the freshness of the raw material. However, the determination of the biogenic amines as a proportion of the water soluble protein indicates the age or quality (and hence freshness) of the solubles. It is believed that addition of formaldehyde to a meal, by binding the biogenic amines, renders analysis of the amines impossible.

9. TOTAL VOLATILE NITROGEN (TVN) IN THE MEAL.

The method measures ammonia plus other volatile nitrogenous bases such as trimethylamine and dimethylamine. Although TVN is used as an index of freshness in the raw material, the amount remaining in the meal is approximately 35 to 65% of that in the raw material. Variable amounts are lost depending on

the drying conditions. The determination has little value in determining either freshness or nutrient or toxic components.

10. DILUTE PEPSIN DIGESTIBILITY AND MINK DIGESTIBILITY

The AOAC method using 0.2% pepsin fails to descriminate between different quality meals. Olley and Payne (1967) showed 0.0002% pepsin solubility of N of fish meal correlated with rat Net Protein Utilization (r=0.93). In a series of Norwegian trials where temperature of processing of fresh raw material was controlled to between 50 C and 140 C, the dilute pepsin solubility was highly correlated with mink true digestibility (r=0.73). The residual standard deviation of a predicted mink digestibility was +/-1.21 digestibility units. Where stale raw material was used (TVN 100 mg N/100g) the pepsin solubility was higher than mink digestibility and did not reflect damage induced by drying such material at high temperature.

As discussed in Sections 2.2 and 2.3, the Norwegian trials with experimental meals produced under controlled laboratory conditions found heating at temperatures above 80 C resulted in reduced N digestibility in mink. However, even at 140 C the reduction in digestibility was only 3.3 percentage units of 3.5% less than fish meal processed at 60 C. Changes in pepsin solubility were usually correspondingly small. In contrast, such extremes of heating produced a 12% decrease in **growth** rate of mink. An even smaller temperature difference (60 C v 110 C) resulted in a 25% decrease in salmon growth. Similarly, as discussed in Section 3, large increases in growth of salmon (5-30%), early weaned pigs (23%), mink (6-19%) and trout (10%) were achieved when commercial Norse-LT 94 was compared with Norseamink while mink digestibility and pepsin solubility differed by only about 5% units. Therefore, both mink digestibility and pepsin solubility are rather insensitive tests of the improvement in nutritive value and animal growth that appears to be achieved with low temperature processed meals under Norwegian conditions.

11. MULTI-ENZYME DIGESTIBILITY AND PH STAT METHOD

Normal digestion involves exposure of protein to a large number of proteolytic enzymes. Attempts to simulate this have been made incubating test proteins *in vitro* with up to three proteolytic enzymes. During the course of digestion the release of amino acids reduces pH of the medium and can influence subsequent enzyme activity. In the pH stat method, pH is maintained constant by continuous titration and the extent of digestion can be estimated from the amount of alkai added. A further advantage of the method is that *in vitro* digestion is followed for only 10 minutes. The titre after 10 minutes has been correlated with rat true digestibility of the N and regression equations are used to calculate an estimated rat digestibility.

In the original work of Pedersen and Eggum (1983) a single regression equation applicable to all feeds of animal or plant origin was proposed. The correlation coefficient between titre and rat digestibility data on which this regression was based was 0.96 with a residual standard deviation of a predicted rat digestibility of 1.29. There was some indication of different relationships for animal compared with plant protein sources but a common equation of TD + = 76.14 + 47.77B10 was preferred. (B10 is the amount of titrant added in 10 minutes).

Beames et al (1987) reported additions of minerals to wheat and soya increased proteolysis and predicted digestibility, probably by increasing the activation of the enzymes. Mann (1988) found it necessary to develop separate regression

equations for each class of plant feedstuff, the correlation coefficients ranging from 0.74 for grains to 0.93 for soybean meals.

In the Danish study (1983), the regression equation used is not cited. The correlation coefficient between estimated digestibility from the pH stat titre and rat true digestibility is reported as 0.6 for 16 experimental meals, omitting one grossly overheated sample. This is much poorer than relationships reported by others for this method. Calculation of the Spearman Rank correlation indicates the fish meals were similarly ranked by the two methods ($P<0.01$) but clearly the precision achieved in the Danish study is inadequate. Meals prepared from stale material (TVN 120 to 250) gave overestimates of rat true digestibility by the pH stat method. Similarly, the value of such meals for growth of early weaned pigs, mink and trout was overestimated. As with pepsin solubility, the test appears to work best when raw material is known to be fresh.

12. DYE BINDING CAPACITY (DBC)

The use of this method as an indicator of amino acid composition was discussed in para 5 above. The method also reflects decreases in availability of lysine as measured in chick bioassays (Barlow et al, 1984). In Norwegian studies comparing low temperature meals with normal processing temperature no difference was found in DBC. Earlier studies have shown that fish meals need to be heated in excess of 115 C for several hours to bring about significant decrease in reactive lysine as a result of intra protein binding with the amide group of glutamine. The method appears to be inappropriate to detect changes in protein structure occurring at 70 to 90°C which result in decreased digestibility and growth in salmon, mink and early weaned pigs.

13. SULPHYDRYL AND DISULPHIDE BONDS

Opstvedt et al (1984) demonstrated that heating fish for 20 minutes at temperatures ranging from 40 to 115 C resulted in a linear decrease in the content of -SH (sulphydryl) groups and a concomitant increase in the content of S-S (disulphide) bonds from 50 to 115 C. At 95 C, the reaction was rapid and had reached equilibrium in 20 minutes. These results were confirmed in the recent Norwegian studies -SH as a % of the total -SH + S-S/2 decreasing from 56% to 19% as processing temperature increased from 60 to 140 C. %-SH was significantly correlated ($r=0.78$) with mink true digestibility of the N. The residual standard deviation of a predicted mink digestibility was 1.18 units. Despite the greater change in %-SH than either mink digestibility or pepsin solubility, the correlation coefficient and residual standard deviation are very similar to those of the relationship between pepsin solubility and mink digestibility. However, the correlation coefficient between pepsin solubility and %SH was 0.87, indicating that variability in the mink assay may limit the development of any closer relationship.

With five experimental meals, salmon growth decreased linearly with decrease in %-SH groups below about 50%. No values are reported for commercial Norse-LT 94 versus Norseamink. Further work is required to test this method with commercial samples and to correlate with animal performance in salmon, trout, mink and early-weaned pigs at various stages of growth. Little appears to be known of how S-S bonds are digested. Work should be undertaken to study the effect of variation in %-SH on rate, site and extent of digestion of protein in the various farmed species. An understanding of this aspect of digestion may lead to alternative ways of maintaining critical protein structures during processing and thereby achieve high nutritive value while processing rapidly at normal temperatures.

Appendix Table 1

DRYING CONDITIONS FOR SELECTED DRIERS PROCESSING SOLID MATERIAL

Drier	Heat Transport Medium	Inlet Gas Temp. °C	Outlet Gas Temp. °C	Heating Surface Temp. °C	Pressure Bar	Evaporation Temp. °C	Drying Time Min
"GAS DRIERS"							
Rotary Drum Drier	Flue Gas	750-500	80-120	-	CA. 1	60-90	10-20
Hot Air Drier	Air	500-200	60-100	-	CA. 1	60-90	10-20
Flash Drier	Air	750-200	80-150	-	CA. 1	60-90	CA.5-10^{-2}
Fluid Bed Drier	Air	300-100	50-150	-	CA. 1	60-90	5-20
"CONTACT DRIERS"							
Disc Drier	Heating Surface	-	-	120-180	CA. 1	85-97	40-120
Tube Drier	Heating Surface	-	-	120-180	CA. 1	85-97	40-120
Drum Drier	Heating Surface	-	-	120-180	CA. 1	50-80	40-120
Vacuum Drier	Heating Surface	-	-	80-150	0.1-0.5	50-80	40-120
Freeze Drier	Heating Plates	-	-	Max 50	10^{-4}-10^{-3}	-40 - -20	240-480

Appendix Table 2

CHEMICAL RAT IN VIVO, AND CHICK GROWTH ASSAYS ON DANISH FISH MEALS

Fish Meal Code	1-2403	2-2503	9-3007	6-1702	6-1802	3-0608	8-0904	12-2702
CHEMICAL ASSAYS								
TVN in raw material (mgN/100g fish)	60	70	60	60	120	140	70	250
pH stat	97.7	90.0	85.8	92.1	93.1	85.5	94.4	91.4
Titration value (mg NaOH)	64.9	65.3	74.6	65.8	87.3	112.6	70.1	103.0
RAT ASSAY								
BV	90.7	90.6	83.8	83.7	80.9	76.2	90.5	85.3
Available Lysine	80.2	83.9	79.9	79.6	77.6	77.0	85.1	80.7
Available Meth+cystine	90.7	90.6	83.8	83.7	80.9	76.2	90.5	85.3
CHICK ASSAY								
Trial 1 Growth (weight (g) at 28 days)	969^{c}	1004^{ab}	1000^{ab}	1014^{a}	995^{ab}	981^{bc}		
Feed conversion	1.53^{a}	1.51^{b}	1.52^{ab}	1.52^{ab}	1.53^{a}	1.53^{a}		
Trial 2 Growth (g/day)	567^{bc}	618^{ab}	605^{ab}	614^{ab}	645^{a}	619^{a}	624^{a}	549^{c}
Feed Conversion	2.12	2.04	2.18	1.99	2.00	2.02	2.05	2.22

(a,b,c values with the same superscripts are not significantly different [P>0.05])

Appendix Table 3

CHEMICAL AND TROUT GROWTH ASSAYS ON DANISH FISH MEALS

Batch No.	Raw mat.	Mg TVN 100 g	Meal Type	pH Stat	Note	Trout Growth
2	Sprat	≤ 30	Pre-mel	85.2	Light	2.02
3	Sprat	≤ 30	Pre-mel	-	Dark	0.25
5	Mackerel	≤ 20	Pre-mel	89.1	Formalin treated	1.74
8	Pilchard	> 250	Whole meal	91.4		1.77
13	Sprat	≤ 40-50	Whole meal	97.7		2.07
14	Whiting	≤ 60-70	Whole meal	90.0		2.13
15	Sprat	≤ 50-70	Pre-mel	97.7	Vacuum-dried	2.00
16	Blue whiting	≤ 70-80	Whole meal	94.4		1.92
17	Sprat	≤ 40	Pre-mel	85.8	250 ppm ethoxyquin	2.29
18	Sprat	≥ 140	Whole meal	85.5	Formalin treated	1.83

Appendix Table 4

QUALITY STANDARDS FOR NORSEAMINK AND NORSE-LT 94

Raw material -	Norseamink No preservative 90mg TVN/100g	Norse-LT 94 No Preservative 50mg TVN/100g
Meal		
Protein	>70%	>60%
Moisture	>5% <10%	>5% < 10%
Fat, Soxhlet	<11.5%	<11.5%
Ash - salt	<14.0%	<14.0%
Ammonia-N	<0.18%	<0.16%
Salt	<3.0%	<3.0%
Pepsin soluble protein, %		>94.0%
Dimethyl nitrosamine	N.D.	N.D.
Salmonella	N.D.	N.D.
Added antioxidant (ethoxyquin)	400 ppm	400 ppm[1])

1) 200 ppm added before and 200 mg added after the dryer

N.D. Not detected.

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