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UTILISATION OF FISH SILAGE BY RUMINANTS

A thesis submitted to the University of Glasgow
for the degree of Doctor of Philosophy
in the Faculty of Science

by

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October 1985

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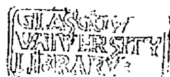

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ABBREVIATIONS

A	=	Abstract
ADAS	=	Agricultural Development and Advisory Service
ARC	=	Agricultural Research Council
CP	=	Crude Protein
DCP	=	Digestible Crude Protein
DE	=	Digestible Energy
dg (or P)	=	Rumen Degradability of Protein
DM	=	Dry Matter
DMI	=	Dry Matter Intake
DOM	=	Digestible Organic Matter
FCE	=	Feed Conversion Efficiency
FFA	=	Free Fatty Acid
GE	=	Gross Energy
IV	=	Iodine Value
k or kr	=	Passage Rate from the Rumen
k_f	=	Efficiency of Utilisation of ME for Gain
LW	=	Live Weight
LWG	=	Live Weight Gain
MAFF	=	Ministry of Agriculture, Fisheries and Food
M/D	=	ME Concentration in the Dry Matter
ME	=	Metabolisable Energy
NAN	=	Non-Ammonia-Nitrogen
ND	=	Not Determined
NH_3-N	=	Ammonia Nitrogen
NPN	=	Non-Protein-Nitrogen
NRC	=	National Research Council
NS	=	Not Significant ($P < 0.05$)
*	=	Significant at 5% level ($P < 0.05$)
**	=	Significant at 1% level ($P < 0.01$)
***	=	Significant at 0.1% level ($P < 0.001$)
RDP	=	Rumen Degradable Protein
TP	=	True Protein
UDP	=	Dietary Undegradable Protein
VFA	=	Volatile Fatty Acids

SUMMARY

1. The literature in the following areas is reviewed: ruminant protein rationing systems and methods of measurement of dietary protein degradability; rate of passage through the ruminant digestive tract; protein supplementation of diets for growing cattle; production and utilisation of fish silage and protection of dietary protein by formaldehyde treatment.

2. Eleven acidified fish silages were prepared using a range of formalin levels (0-40 cm^3 kg^{-1} fish waste). White fish filleting scrap was minced and well mixed with formic acid (1.5-2.0% v/w) before the addition of the appropriate level of formalin. The silages were stored before analysis in closed plastic containers at a temperature of 23 ± 3°C for 10 days. Treatment with 10, 20, 30 and 40 cm^3 formalin kg^{-1} waste yielded a hard rubber-like product unsuitable for animal feeding. Lower levels of formalin (1-5 cm^3 kg^{-1} waste) gave a slurry-like material. Formalin addition led to a linear increase in the proportion of true protein (as % of crude protein) from 13% for untreated silage to 41% for fish silage treated with 5 cm^3 formalin kg^{-1} waste.

3. The Dacron bag technique described by Ørskov and Mehrez (1977) was used in an attempt to estimate the effect of formalin addition on rumen breakdown of fish silage protein. Formalin treatment of fish silage significantly ($p < 0.05$) reduced the losses of DM and N from the bags. However, this technique was considered a poor guide to the degradability of soluble fish silage protein because it is based on the faulty assumption that all the material leaving the bag is completely degraded.

4. An _in vitro_ procedure developed by Broderick (1978) was used in an attempt to measure the rate of degradation of untreated fish silage, formalin treated fish silage and a number of other protein sources. The rate of degradation and proportion of protein escaping ruminal degradation was estimated from the

release of amino acid plus ammonia in the presence of hydrazine sulphate which inhibits amino acid deamination and ammonia utilisation by rumen micro-organisms.

The technique ranked casein, bovine serum albumin, soya meal and fish meal in the same order as obtained by other workers using a range of methods. However, it proved unsuitable for estimating the rate of degradation of fish silage since amino acid deamination was totally inhibited and fish silage contains high levels of free amino acids.

5. Alternative inhibitors for ammonia utilisation by rumen micro-organisms were tested to replace the hydrazine sulphate. Those tested were penicillin G, glutaric acid and m-bromobenzoic acid.

   The highest recoveries ($p < 0.05$) of ammonia were obtained with glutaric acid.

6. Further _in vitro_ incubations of untreated fish silage and formalin treated fish silage were conducted using glutaric acid instead of hydrazine sulphate. This allowed ammonia accumulation to be used as an index of degradation rate. Formalin addition led to a linear decrease in rate of ammonia production. At 5 cm^3 formalin kg^{-1} waste the total production after 5 hr incubation was 59% of that for untreated fish silage. Untreated fish silage gave a rate of ammonia production similar to that obtained for casein. Treatment with 5 cm^3 formalin kg^{-1} waste gave a rate which was approximately double that measured for fish meal.

7. Four tonnes of fish silage were manufactured for use in a calf feeding trial, a passage rate trial and a digestibility trial. Two types of silage were prepared by mincing white fish waste and adding either 25 kg t^{-1} formic acid or a mixture of 25 kg t^{-1} formic acid + 5 kg t^{-1} formalin. The product was mixed thoroughly and allowed to mature over a period of 14-20

days at a temperature of 20°C before feeding.

8. A feeding trial was conducted to test the inclusion of untreated and formalin treated fish silages as protein supplements for young steer calves. The basal diet consisted of barley (approximately 573 g kg^{-1}) and barley straw (222 g kg^{-1})) molasses (87 g kg^{-1}) and mineral/vitamin mixture (46 g kg^{-1}) fed as a complete diet _ad libitum_. Urea and white fish meal were used as negative and positive controls respectively. The five diets were found to contain the following levels of crude protein and UDP respectively (g kg^{-1} DM): diet CS (untreated silage) 127 and 27; diet FS (formalin treated fish silage) 128 and 29; diet CSFM (half fish meal, half untreated fish silage) 129 and 33; diet U (urea) 134 and 16, and diet FM (white fish meal) 134 and 41. All diets were adequate in rumen degradable protein according to ARC (1983).

   Thirty Friesian steer calves, average weight 79.0 kg and age 10 weeks at the start of the trial were allocated to the five experimental diets in a randomised block design. The duration of the trial was 15 weeks.

   Animals on diets CS, FS, CSFM, U and FM consumed 3.44, 3.68, 3.96, 4.16 and 4.09 kg DM d^{-1}. The average live weight gains (LWG) were 0.87, 0.92, 1.08, 1.04 and 1.13 kg d^{-1} and the average feed conversion efficiency ratios (FCE) were 4.27, 4.37, 4.01, 4.28 and 3.87 kg DMI per kg LWG. Significant differences in DMI, LWG and FCE were recorded.

   Over 15 weeks, intake and performance were depressed by the incorporation of fish silage in the diet. Formalin treatment of fish silage slightly improved these parameters. For the latter half of the trial, the reduction in intake and performance for the calves receiving fish silage became less. Live weight gains for this period were similar for the urea and fish silage treatments but fish silage supported a better FCE than urea ($p < 0.05$) although inferior to fish meal.

9. Five mature wethers were allocated to a 5 x 5 Latin square design to assess the effect of fish silage (and formaldehyde treatment) on diet digestibility. The five experimental diet used in the feeding trial were tested.

   There were no significant differences ($p < 0.05$) in dry matter, organic matter or energy digestibility.

10. Six Friesian steer calves, age 24 weeks, were allocated to a cross over design to measure passage rates. The soluble marker Cr-EDTA was used to measure liquid passage rates (applicable to fish silage) and compared with the passage rate of Cr-treated fish meal as a particulate insoluble marker. The calves were fed the ration CS (basal diet supplemented with untreated silage). The markers were administered orally in a single dose 1 hr after feeding and the decrease with time in concentration of Cr in faecal dry matter was measured.

    The mean passage rates of Cr-EDTA and Cr-treated fish meal were 0.079 and 0.052 hr^{-1} respectively. The difference was highly significant ($p < 0.01$) and the implication of this finding to the degradability of liquid protein supplement is discussed.

11. The practicality and economic feasibility of feeding ruminant stock on fish silage produced from fish waste is discussed.

CHAPTER ONE

REVIEW OF LITERATURE

GENERAL INTRODUCTION

There is need for improvement in the output of meat, milk and milk products throughout the world to meet the demands of increasing human population and standards of living. The ruminant's ability to digest various waste products must be exploited if this is to be achieved.

There has been renewed interest in fish silage in several parts of the world as a means of utilising fish waste where intermittent availability of only small quantities of waste fish may make the production of fish meal commercially unattractive.

Feeding trials with fish silage have shown that it can be used to replace conventional protein supplements such as fish meal or soya bean meal in the diet of pig and poultry. There is little known about its nutritional value for ruminant animals. However, the work of Tatterson and Windsor (1974) and Gildberg and Raa (1977) on the chemical composition of acid preserved fish silage suggested that its high soluble nitrogen content might limit its value as a protein supplement for ruminants.

The object of this work is to evaluate the use of fish silage as a protein supplement for ruminants. The investigation was carried out in two stages:

A) *in vitro* evaluation

1. laboratory scale production of fish silage and chemical analysis;
2. an attempt to improve the protein quality of fish waste by formaldehyde treatment;
3. the development of a quick *in vitro* technique to assess the rate of degradation of fish silage protein;
4. comparison between *in vitro* degradation of fish silage and other protein sources.

B) *in vivo* studies

1. on-farm production and handling of fish silage;
2. feeding trial with growing calves, to compare fish silage with other protein supplements;
3. digestibility trial with sheep to study the effect of fish silage on diet digestibility;
4. measurement of solid and liquid phase passage rates in calves fed fish silage supplemented diet.

SECTION 1

PROTEIN RATIONING SYSTEMS FOR RUMINANTS

The expression of protein requirement for ruminants, and the protein value of feedstuffs to satisfy this requirement, in terms of crude protein (CP) or digestible crude protein (DCP), has been used for many years. However, CP and DCP have been regarded as unsatisfactory for a long time. Neither of these parameters recognise the close relationship between nitrogen requirement and either the total energy intake or energy density of the ration fed. Also, neither system gives an adequate description of the dietary nitrogen, nor the role of rumen microbes in its digestion.

Many experiments have shown that diets with equivalent DCP content do not necessarily sustain the same productivity (Whitelaw, Preston and Macleod, 1964). Even under isoenergetic, isonitrogenous conditions it is quite easy to demonstrate differences in animal productivity between different types of protein as supplements to a concentrate (Whitelaw, Preston and Dawson, 1961) or roughage basal diet (El. Shazly, 1958; Little, Burroughs and Woods, 1963). In an attempt to improve on the CP and DCP systems, an alternative approach based on available protein (AP) was proposed (ARC, 1965). The AP system still did not consider protein - energy interrelationships, nor the large contribution made by undigested microbial protein to faecal nitrogen loss. The AP system can be further criticised for the use of biological values to describe dietary proteins which are not relevant to the ruminant. This is because the composition, and hence biological value, of the ingested protein is changed by rumen degradation and subsequent incorportation into microbial protein.

Several new protein rationing systems have been proposed in the latter half of the 1970s (Burroughs, Nelson and Mertens, 1975; Journet and Verite, 1977; Kaufman, 1977; Roy *et al.*, 1977; Satter and Raffler, 1977). These systems all incorporated the basic ideas that the supply of N to the animal should be assessed in terms of the amino acid N absorbed from the small intestine and that the diet formulation should take account of the N needs of the rumen microbes as well as those of the animal. The details of the various systems

differ considerably but in each there are components to allow for the partial degradation of dietary protein in the rumen and the ruminal synthesis of microbial protein.

The British system (Roy et al., 1977) has now been fully described in ARC (1980). According to this system the protein metabolism of ruminants is regarded as consisting of two sub-systems:

(a) A rumen microbial sub-system with a N requirement and producing microbial protein, and

(b) a host animal sub-system having a requirement for true protein synthesised in the rumen and for highly productive animals an additional requirement for undegraded dietary protein.

The nutritive value of protein in a feedstuff is measured in terms of its rumen degradable protein (RDP) and rumen undegradable dietary protein (UDP).

Other factors, such as efficiency of utilisation of absorbed amino acids are important to the absolute estimate of protein requirement, but microbial yield and dietary protein degradability are the factors which vary most with diet composition. In general the microbial yield is reasonably predictable from digestible organic matter content of feed. Also the digestion in the small intestine is reasonably constant, therefore, the main variable affecting the nutritive and economic value of feedstuff protein is the degradability of the protein in the rumen.

The new system has been put into practical application by some agricultural development agencies and by the food trade with minor modifications. However, the system has unresolved limitations to its complete adoption, for example, the methodological problems of estimating dietary protein degradation in the rumen. Some of these limitations have been reviewed by Thomas and Chaimberlain (1982).

Measurements of Feedstuff Protein Degradability

Several methods have been developed in an attempt to measure dietary protein degradability in the rumen. All these methods show some promise but none have provided a reliable technique. All methods are subject to considerable error. Table 1 shows that, even when a particular laboratory uses the same sample of feedstuff, different techniques can give widely differing degradability value. However, some workers have achieved similar values using different methods. Therefore, a method for assessing degradability was required to:

1. give estimated values as close as possible to that likely to be observed under practical feeding situations;

2. have sufficient sensitivity to distinguish quantitatively between raw materials;

3. have sufficient replication over a long time-scale to allow a continuous comparison of raw materials;

4. assess the large numbers of raw materials on offer to the trade (Wilson and Strachan, 1981).

TABLE 1

Comparison of estimates of degradability of dietary protein using different techniques

Feedstuff	Degradability (%)	Method used	Author
Fish meal	35	*in sacco*	Cronje and Mackie (1983)
	81	*in vitro* (diazo protein)	"
Soya bean meal	56	*in vitro* (fungal protease)	Laycock and Miller (1983)
	70	*in sacco*	"
Field beans	56	*in vitro* (funal protease)	"
	44	*in vitro* (*S. griseus*)	"
Grass silage	91	*in vivo* (^{35}S incorporation)	Rooke *et al*. (1983)
	81	*in sacco*	"
Herring meal	50	*in vitro* (*S. griseus*)	Cottril (1983)
	45	*in sacco*	"
Lucerne	72	*in vivo* (^{35}S incorporation)	Mathers and Miller (1981)
	73	*in sacco*	
Barley	86	*in vivo* (^{35}S incorporation)	"
	86	*in sacco*	"

I - *in vivo* Methods

Several techniques have been developed to measure dietary protein degradability *in vivo*. The methods require two sets of measurements:

(1) *in vivo* estimation of total passage of protein to the duodenum. This requires the collection of samples representative of the digesta passing to the duodenum and the measurement of flow rates. Animals fitted with simple or re-entrant connula (Hogan and Phillipson, 1960; Axford *et al.*, 1971) and indigestible markers have been used for this purpose (Hogan, 1964; Nicholson and Sutton, 1969). The techniques present considerable technical difficulties and results in a high degree of error (see reviews by Smith, 1975; Stern and Hoover, 1979). MacRae (1975) has reviewed the problems inherent in the use of surgically modified animals.

The majority of determinations have been made with sheep at the maintenance level of feeding. A few studies have been made with lactating cows because it is extremely difficult to make determinations on cattle consuming large amounts of feed and reaching top commercial standards of production. Therefore, there are considerable possibilities for error if findings with cannulated animals are extrapolated to commercial animals.

(2) the partition of the protein collected in digesta samples into its main components, microbial protein and undegraded dietary protein. Most methods have aimed at measuring the former directly and the undegraded dietary protein by difference.

Techniques for Fractionating Duodenal Digesta Protein

A. Measurement of microbial protein

These methods measure the proportion of microbial nitrogen reaching the duodenum and by difference [from total non-ammonia-N (NAN)] the the proportion of undegraded dietary-NAN. A correction for endogenous N entering the abomasum, and in certain circumstances,

N secreted via the pancreatic and bile ducts, has sometimes been applied. Depending on the nature of the dietary N, microbial N may contribute from less than 50 to over 90% of NAN reaching the duodenum. The amount of microbial-N is more closely determined by the amount of energy released during fermentation in the rumen than by dietary N supply.

Several techniques are available to determine the proportion of microbial-N reaching the duodenum (see recent review by Stern and Hoover, 1979).

1. Naturally occuring markers

Many of the *in vivo* techniques employ a marker which is synthesised by the rumen micro-organism. Metabolites synthesised by the microbial fraction can also be exploited. All techniques require the isolation of a representative pure sample of the microbial population (without contamination), usually using differential centrifugation. This in itself presents problems, often greater than those associated with subsequent chemical analysis. Therefore, some workers used standard calculations from other experiments which introduced inaccurate results.

Use of diamino pimelic-acid (DAPA)

The most widely used natural marker for microbial protein has been α, E-diaminopimelic acid (DAPA). This amino acid is confined to the cell wall glycopeptide of certain gram-negative bacteria, although it is absent from some species. It is not found in protozoa or the usual animal feeding stuffs. Samples are obtained from rumen fluid and compared with the DAPA concentration of the duodenal protein. The proportion of bacterial N in the duodenal digesta NAN is determined from the ratio (DAPA : N in digesta/ DAPA : N in bacteria). The main objection to the use of DAPA is that its concentration varies with species (Work and Dewey, 1953), with diet (Weller *et al*., 1962) and may vary with time, even in animals given a constant diet (Allen, 1971). This means that the ratio, DAPA : N of the bacterial fraction must be determined for each experimental situation. Another potential problem of this technique is the possibility of metabolism of DAPA prior to the digesta

sampling. Mercer et al. (1980) have found the DAPA : N ratio in digesta samples to be greater than that of bacterial preparations. This error results from the turnover and lysed bacteria in the rumen DAPA is released which is resistant to further degradation in the rumen and passes with the digesta to the duodenum and this could lead to an over-estimate of the microbial yield. DAPA is not a constituent of protozoal protein so this method does not take into account the contribution made by protozoa. Protozoal protein flow can be assessed using aminoethylphosphoric acid (AEPA) or phosphatidylchlorine.

To make an accurate estimate of bacterial protein at the duodenum it is essential that the diet contains no DAPA and that DAPA is found only in bacteria at a constant ratio. However, Czerkawski (1974) found that the diet may contain traces of DAPA and that it can be detected in protozoa, probably due to the engulfment of bacteria.

Perhaps the major advantage of the DAPA technique is that it is relatively easy to assay since it shares with proline the property of giving a yellow colour with acid ninhydrin This has enabled simple ion-exchange chromatographic systems to be developed. The method has been described in more detail by Hutton et al. (1971) and Harrison et al. (1973).

Use of aminoethylphosphoric acid (AEPA)

Attempts have been made to estimate the protozoal contribution using 2-aminoethylphosphoric acid (AEPA) as the marker. This amino acid is found in the lipid fraction of protozoa and represents one of the few methods of determining the protozoal contribution to duodenal protein flow. Unfortunately it is difficult to assay. Although Abou Akkada et al. (1968) determined the phosphate content of AEPA by a mixture of paper and column chromatography, the method is time consuming and tediuous. Czerkawski (1974), again using column chromatography, estimated AEPA by determining phosphate following acid hydrolysis. Ling and Buttery (1978) developed a satisfactory system using cation-exchange chromatography on a conventional automatic amino acid analyser in which AEPA was eluted towards the start of the chromatogram. For the technique to give accurate

results it is essential to ensure the use of relatively concentrated hydrolysates to detect the AEPA.

Although it has been reported that AEPA was absent from feed, Abou Akkada et al. (1968); Ling and Buttery (1978) and Czerkawski (1974) have found that AEPA occurs in both bacteria and feedstuffs as well as protozoa. Therefore, this technique requires careful use and due to the large corrections required to allow for dietary AEPA it is far from satisfactory.

Use of phosphatidylcholine (PC)

An alternative protozal marker is phosphatidylcholine (PC) reported by John and Ulyatt (1979). Although PC may also occur in small quantities in feedstuffs, studies so far indicate that this is degraded in the rumen and that PC reaching the duodenum is mainly of protozoal origin. Radio-active labelling studies indicate there may be some endogenous secretion of PC into the abomasum

Use of Nucleic acid

This technique is based on the assumption that all dietary nucleic acids (RNA and DNA) are largely degraded in the rumen (McAllan and Smith, 1968), and there is a constant proportion of nucleic acid in the microbial N reaching the duodenum. There is some doubt about this assumption and microbial protein flow may be over estimated. This may be particularly important when large portions of the dietary protein and nucleic acid have been rendered insoluble by exposure to heat or chemical treatment. For this reason microbial estimates may be unreliable when animals are fed protein supplements of animal origin, much of which can pass from the rumen undegraded.

Ellis and Pfander (1965) investigated the DNA and RNA content of rumen fluid incubated in vivo. They concluded that nucleic acids formed 15% of the total microbial nitrogenous compounds produced. The ratio of DNA and RNA to total nitrogen was determined for mixed bacterial cultures obtained from the rumen of sheep fed a variety of different rations. The RNA/total nitrogen ratio was relatively constant, and the quantity of RNA in duodenal digesta has been used

as an indication of microbial protein content. By comparing a mean value for RNA/total nitrogen in rumen organisms with mean values for this in samples of calf digesta obtained 4 hours after feeding, Smith (1969) estimated that, for most diets, about 70 and 60% of the total-NAN in ruminal and duodenal digesta respectively was microbial. This method has been used by several workers and discussed in more detail by Smith (1975). In order to overcome the analytical problems Ling and Buttery (1976) adopted the much simpler method of Guinn (1966) so that it was suitable for rumen digesta samples. The method was based on extraction of nucleic acid fractions and subsequent assay of the RNA using orcinol. In addition, the effect of DNA on the orcinol reaction could be corrected by determining the DNA content using the diphenylamine reaction.

RNA is the preferred marker, since it is less variable and gives more appropriate weighting for both bacteria a protozoa (Smith, 1975). The RNA : N ratio of bacteria is also influenced by diet and by time after feeding (McAllan and Smith, 1974). Therefore, average RNA : N content of digesta and microbial preparations must be determined simultaneously for each animal and diet combination.

There are also a number of chemical methods for determining RNA, and these do not always give similar values (Ling and Battery, 1978; Smith and McAllan, 1974).

The RNA method has the advantage over the DAPA method in that protozoal nitrogen will be included. McAllan and Smith (1974) compared RNA and DAPA as markers for determining the contribution of microbial nitrogen in duodenal digesta. Experimental animals included a protozoa-free calf and a faunated cow. They found good agreement between methods for the protozoa-free animal; however, when the faunated cow was used they found a marked discrepancy between the two techniques. The microbial nitrogen to total NAN ratios in duodenal contents in the faunated cow were 0.78 and 0.40 for the RNA and DAPA methods, respectively. Unless considerable amounts of dietary RNA survived rumen degradation in the cow experiment, it seems possible that the DAPA method under-estimated the microbial contribution in the cow because it did not account for the presence of protozoa. This nucleic acid method does not require

expensive apparatus, but has the disadvantage that it assumes the food eaten is either relatively free of RNA, or that the RNA from feed is degraded in the rumen.

2. Isotopic labelling

The use of isotopes is probably the most reliable method for determining microbial yield when isotopes are correctly used (Siddons et al., 1979). The most widely used isotope has been ^{35}S but ^{14}C, ^{32}P and ^{15}N have also been used.

^{35}S incorporation into microbial protein

The technique depends on labelling of microbial fraction with ^{35}S, usually $^{35}SO_4^{2-}$ and then the determination of the dilution of the specific activity of sulphur component in both digesta and the microbial fraction.

Use of ^{35}S as a microbial marker was first suggested by Hendrikx (1961). The method of using ^{35}S as a microbial marker has been to measure differences in the ratio of specific activities of ^{35}S in either the cysteine (Leibholz, 1972), methionine (Beever et al., 1974) or total sulphur amino acids (Hume, 1974) of duodenal digesta and a separated microbial fraction.

Beever et al. (1974) have reported that methionine is more likely to be directly incorporated into protozoal protein and that the ratio of protozoa to bacteria may be lower in the isolated microbial fraction than that in the rumen, and specific activity of microbial fraction would be over-estimated with a reduction in estimated contribution of microbial protein to digesta. But the same workers suggested that specific activities were similar when the infusion of $^{35}SO_4^{2-}$was carried on for long enough.

Walker and Nader (1975) described an in vivo method which depends upon the incorporation into microbial protein of sulphur derived from ^{35}S-labelled inorganic sulphate infused continuously into the rumen. This method has the advantage of not relying on the use of highly labile sulphide and does not require that virtually all microbial sulphur be derived from hydrogen sulphide. They compared estimates

of microbial protein synthesis using the above ^{35}S method with values obtained using DAPA from 27 individual experiments. They found a significant correlation (r = 0.68) between the two methods, with DAPA estimates averaging approximately 30% less than ^{35}S estimates. This was attributed to the inability of DAPA to account for protozoal protein.

However, the ^{35}S technique is relatively expensive and laborious for routine screening of diets. It suffers from a failure to allow for the effect of endogenous protein. The differences in the ratio of specific activities of ^{35}S in sulphur amino acid in digesta and microbial fraction may not be completely accurate.

Mathers and Miller (1980) developed a simple procedure which has less bias, a lower coefficient of variation, and is suitable for routine analysis. Animals are given $^{35}SO_4^{2-}$ either by intraruminal infusion or by the addition to the feed given at 2 hr intervals. On the 4th-6th day total collection of duodenal digesta were obtained twice a day for periods of 0.5-1 hr from a single cannula. A microbial pellet is prepared from the duodenal digesta by differential centrifugation. Freeze-dried digesta and microbial isolates are treated with performic acid to convert contaminating sulphide to sulphate, and removed by precipitation with barium chloride. The radioactivity and N content of the hydrolysate are determined and the ratio ^{35}S : N is calculated. Freeze-dried digesta are also analysed for ammonia, N and chromic oxide. The flow of NAN and the flow microbial N were calculated and, for sheep, the endogenous N contribution is assumed to be 1.5 g N d^{-1} (Harrap, 1974). The undegraded dietary N is then calculated by difference. The degradabilities were determined for the whole diet which consisted either of all lucerne or barley or mixtures of the two.

The use of ^{35}S is preferred for reasons of safety and ease of use. Quantitative recovery of the sulphur amino acids is not essential, as the technique is based on a ratio of specific activities. Also, the technique is capable of determining total microbial protein synthesis rather than just bacterial protein synthesis as with the DAPA technique.

N incorporation into microbial protein

Microbial protein synthesis has also been estimated by quantifying ^{15}N incorporation into microbes from either $(^{15}NH_4)_2SO_4$ (Pilgrim et al., 1970) or $^{15}NH_4Cl$ (Mathison and Milligan, 1971). The one great advantage of this technique is that it deals directly with nitrogen, and it has proven to be a useful tool in studying the dynamics of nitrogen metabolism in the ruminant (Nolan et al., 1976).

Mathison and Milligan (1971) infused $^{15}NH_4Cl$ for a period of 120-216 hr into the rumens of sheep given either a barley diet or one of three hay diets. Lignin and polyethyleneglycol (PEG) infused into the rumen were used to estimate the rate of flow through the abomasum. The concentrations of ^{15}N in the nitrogen of urine, faeces, rumen and abomasal bacteria and protozoa, rumen ammonia and abomasal particulate matter were determined. Comparison of the concentration of ^{15}N in the microbes with that in the rumen ammonia indicated that from 50-62% of bacterial-N and 31-55% of protozoal protein was derived from rumen ammonia. Between 60-92% of the dietary-N was transformed into ammonia in the rumen and 17-54% was absorbed directly.

Nolan and Lang (1972) proposed a quantitative model for nitrogen pathways in the sheep using isotope dilution techniques. ^{15}N ammonium sulphate, ^{15}N urea and ^{14}C urea were administered both by single injection or by continuous infusion into the rumen. Sheep were fed 33 g lucerne hay hr^{-1} providing 23.4 g N d^{-1}. It was estimated that 59% of the dietary N was utilised as amino acids by micro-organisms and 71% was degraded to ammonia. Of the 14.2 g N d^{-1} entering the ruminal ammonia pool 9.9 g N d^{-1} did not return, the difference of 4.3 g N d^{-1} represented recycling largely within the rumen.

Kennedy and Milligan (1978) compared the use of ^{15}N with ^{35}S as microbial markers in cold-exposed sheep. Estimates of microbial protein production determined by reference to ^{15}N were 10% lower than estimates made using ^{35}S as a microbial marker. They suggested that the lower estimate from ^{15}N was a possible result of secretion in the omasum or abomasum of organic ^{35}S from endogenous source, or

passage of organic ^{35}S in bacterial oxidates from the rumen.

The disadvantages of these methods is that they are based on the incorporation of nitrogen from ammonia only and do not account for microbial protein synthesised directly from amino acids or peptides. Besides being costly, these technqies are complicated and as a result have not been extensively used.

^{32}P incorporation into microbial protein

Bucholtz and Bergen (1973) observed that phosphorus uptake and incorporation into microbial phospholipids was highly related ($r = 0.98$) to ruminal protein synthesis and they proposed an *in vitro* method for estimating microbial protein production based on incorporation of ^{32}P into microbial phopholipids.

Van Nevel and Demeyer (1977) expanded this approach to include the incorporation of ^{32}P labelled extracellular phosphate in total microbial protein as the measure of microbial growth. This method is based on the following assumptions:

1. all P incorporated into microbial fraction was derived from the labelled precursor pool;
2. the specific activity of the intracellular precursor pool equalled the specific activity of the extracellular pool;
3. there was no degradation of nonlabelled cells;
4. cell composition remained constant during growth.

Unfortunately many *in vitro* studies show that assumptions 3 and 4 are not valid in incubations with rumen micro-organisms. Smith *et al*. (1978) proposed an *in vivo* technique based on the incorporation of ^{32}P labelled inorganic phosphate into rumen bacterial nucleic acid. They compared nucleic acid ^{32}P: NAN ratios in related samples of rumen bacteria and duodenal content with similar estimates using DAP and RNA as bacterial markers. Their estimates based upon ^{32}P-RNA nucleotides were 85% of those based upon total RNA. They attributed this difference mainly to the latter being elevated by the presence of dietary RNA.

3. Use of a protein-free purified diet

If a purified diet is used which contains no protein, there are no complications of distinguishing between feed N and microbial N. If the animals are fitted with post-ruminal cannula, all digesta flowing out from the rumen can be collected to determine the N flow and the ammonia N can be subtracted from this value. The only uncertainty of this method is the assessment of the endogenous contributions of a braded rumen epithelium and enzymes, but it has the advantage that no protein is complicating the measurement (see review by Ørskov, 1982).

B. Measurement of undegraded dietary protein

1. Naturally marked feed protein

A number of naturally occurring markers of dietary protein have been used to partition digesta protein. An earlier experiment by McDonald (1954) has shown that zein is very much more slowly attacked than other proteins in the rumen, and contains no lysine. Advantage was taken of these facts; hence if the dietary zein were used by ruminal micro-organisms, they have to synthesise their own lysine (7%). The difference in lysine contents of the diet and abomasal contents could therefore be exploited to estimate the output of microbial protein from the rumen. Hume (1970) used the alcohol solubility of zein to separate it from microbial and endogenous protein. McDonald and Hall (1957), Williams and Smith (1975) have used the alkali-labile phosphorus content of casein or formaldehyde treated casein, respectively, reaching the duodenum. A chemical procedure was developed for determining small amounts of casein in the presence of other proteins. This procedure relies on the removal of inorganic phosphate from the original sample by dialysis. The alkaline hydrolysis of the phosphate group of casein and the alkali-labile phosphorus (ALP) naturally bound to casein was the marker used to quantify the undegraded casein in digesta. However, these markers are absent from many feedstuffs, so the method is of little general application

2. Use of amino acid profile

McDonald (1954) used the fact that zein contains no lysine, while microbial protein contains approximately 7% lysine, to calculate the proportion of zein in duodenal digesta from its determined lysine content. Evans, Axford and Offer (1975) extended this method to feedstuffs by using not only one amino acid but the whole profile of amino acids in the feed, microbial, endogenous and duodenal digesta protein. It was assumed that the profile of digesta was the weighted sum of the various profiles contributing to it. The method depended upon the use of a computer program which will calculate the composition of the duodenal digesta when given the complete amino acid profiles of the diet, the digesta, the protozoal and bacterial together with estimates of the contribution and composition of the endogenous protein. The method assumes constant composition of microbial protein and that protein in each dietary components behaves as a single entity. This method has the advantage that it relies on the analysis of several different constituents, (the amino acids). Evans et al. (1975) investigated the relationship between total microbial amino acids, estimated by their method, and DAPA passing through the duodenum and found an r value of 0.92 based upon 21 samples.

Buttery and Cole (1977) suggested that this method with more development would yield answers with less bias. However, the method has limitations associated with the estimation of the contribution of endogenous proteins together with problems associated with the effect of the differential degradation of individual proteins found with each dietary constituent (Nikolic and Jovanoic, 1973). Also the amino acid profiles of endogenous secretions and of many feedstuffs (e.g. grass) are not sufficiently different from microbial protein to make an accurate distinction. Siddons et al. (1979) showed that in a comparison of methods of estimating microbial protein, the amino acid profile method gave much lower values of microbial N than of any other method.

Conclusions

Most of the *in vivo* techniques employ a marker which either incorporated into or synthesised by, the rumen micro-organism. Such as, DAPA, AEPA, RNA, and isotopes. Amino acid profiles of different components flowing to the duodenum have also been used.

Estimates of microbial yields for different diets have been very variable, a large part of this variability is probably due to differences in methodology between laboratories (see Table 1).

Several factors have been found to affect microbial growth efficiency, such as diet composition, level of intake, passage rate, NH_3 concentration in the rumen, N : S ratio of diet and protein degradability.

However, even if methodological problems of measuring digesta flow and the contribution of microbial protein are improved, and variation is taken into account by the use of sufficient replicate animals, these methods will not provide a universal estimate of protein degradability. The values relate only to the particular condition used in the trial, especially to the retention time of the dietary protein in the rumen during which it is subjected to microbial attack. All these inherent problems should be resolved in future studies.

Despite their inaccuracies and biases these methods are assumed to provide the reliable values of protein degradability against which other quicker methods, suitable for laboratory or routine use can be calibrated.

Ling and Buttery (1978) reported a comparison between ^{35}S, DAPA, RNA and AEPA techniques and they concluded that the best over-all picture of microbial activity was probably given using ^{35}S technique, although the RNA method is very attractive since it much less complex and is more rapid.

II - *in sacco* METHODS

The *in sacco* technique was first used to assess digestibility when leaves were placed in a perforated brass capsule and fed to sheep (Reaumar, 1752, cited in Wilson and Strachan, 1980).

Quin *et al*. (1938) used material based on silk fibres but this was changed to the artificial fibres which were totally resistant to microbial degradation. Schoeman *et al*. (1972) used the polyester bag to assess the effects of formaldehyde on degradation of protein supplements in the rumen. Ørskov and Mehrez (1977) described the possibility of applying this method to the determination of protein degradability values to be used in the proposed ARC (1980) system.

In this method, several bags were incubated and withdrawn at different times so that a description of degradation rate was obtained. The technique gives characteristic disappearance curves for feed stuffs from which both rate and extent of N disappearance can be calculated by fitting the exponential equation described by Ørskov and McDonald (1979) of the form:

$$P = a + b\,(1 - e^{-ct})$$

where P is the degradation during the time t;

- a - the intercept at time 0, represents soluble N that is rapidly washed out of the bag;
- b - the difference between the intercept and asymptote, represents the insoluble but potentially degradable protein;
- c - the rate at which function b is degraded or solubilised by the rumen micro-organisms assuming first order kinetics.

Miller and Laycock (1983) used the equation above to calculate the rate of N disappearance of three feed stuffs which differ markedly in the extent and rate of disappearance of N (see Figure 1).

It is also necessary to know the passage rate of protein from the rumen (see page 56). Ørskov and McDonald (1979) combined the degradation and rate of passage to give an expression of actual degradability (P or dg):

$$P(dg) = a + \frac{bc}{c + k}$$

where a, b and c are constants from the equation of degradability

and , k is the fractional passage rate.

There are, however, a few substrates where the constant (a) will be negative (e.g. Lucerne, fish meal and soya bean meal). This occurs where there is little or no soluble material and a lag phase before degradation proceeds. McDonald (1981) suggested that, if a is found to be negative, a slight improvement in accuracy can be obtained by introducing another factor, namely a lag phase, and the final formula becomes modified to:

$$P = a \frac{bc}{c + k} \exp(-(c + k)t_o).$$

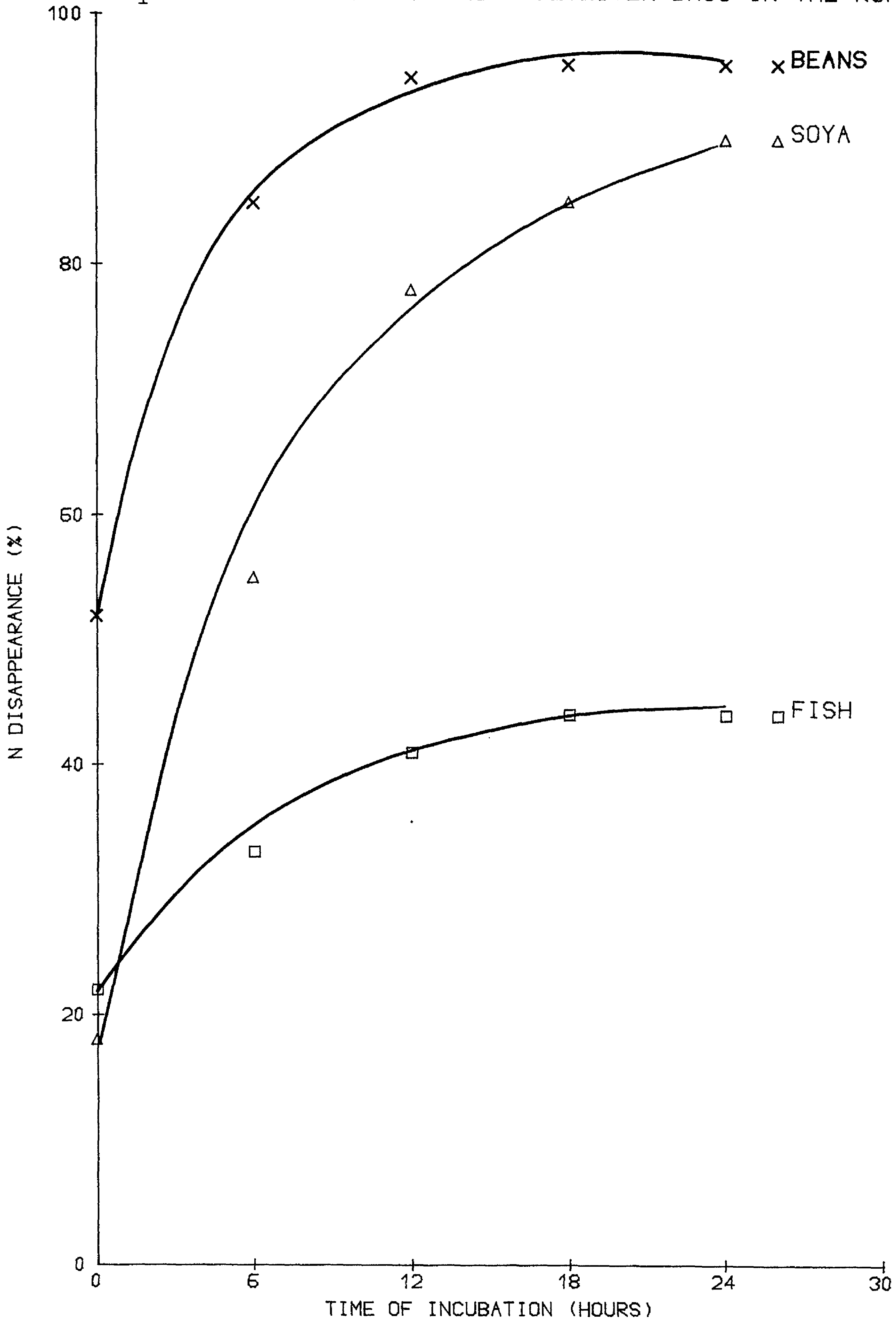
The model is described in detail by McDonald (1981). However, the modification makes little difference to the value of P, and in almost all instances, sufficient accuracy can be obtained using the simple expression:

$$P = a + \frac{bc}{c + k}$$

Factors which affect degradation of feed protein *in sacco*

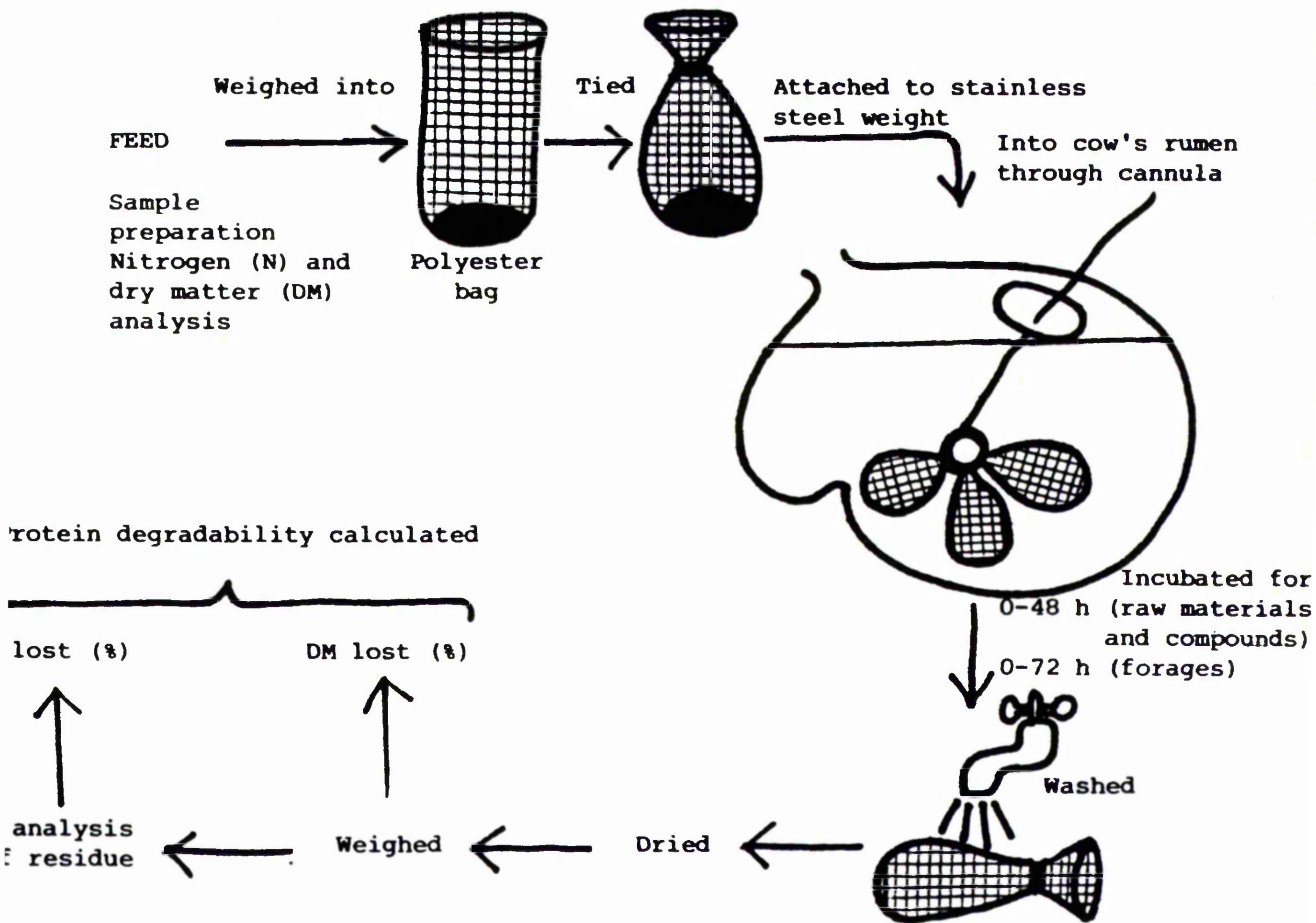
1. The size of bag relating to sample size (Ørskov, 1982)
2. Pore size of the bag material (Mehrez and Ørskov, 1977)
3. Type of substrate fermenting in the rumen of host animal (Mehrez, 1977; Mohammed and Smith, 1977)
4. Sample preparation (Ørskov *et al*., 1980)
5. Time of feeding host animal (Ørskov, 1982)
6. Species differences between cattle and sheep (Ørskov *et al*., 1980)
7. Positioning of bag in the rumen (Mehrez, 1977)
8. Withdrawal times (Ørskov and Mehrez, 1977)
9. Washing technique (Donaldson, Offer and Thompson, 1981)

FIGURE 1 . N DISAPPEARANCE FROM POLYESTER BAGS IN THE RUMEN



*MILLER AND LAYCOCK (1983)

Figure 2: *In sacco* technique for the determination of protein degradability



*Wilson and Strachan (1981)

Description of *in sacco* technique

The technique has been reviewed by Wilson and Strachan (1981), Miller (1982) and Ørskov (1982). Instead of attempts to fractionate duodenal or rumen digesta protein into feed and microbial components the test feed protein is isolated from the rumen digesta in a polyester or nylon bag. The pore size of the bag is such that rumen fluid and bacteria pass through easily but insoluble dietary protein is retained. After fixed times of incubation, the bags are taken from the rumen, washed with running water to remove adhering bacteria and the residual dietary N is determined (see Figure 2). The bag must be used with care as there are feed materials for which it is not suitable. Also, it must allow escape of accumulated gas. Accumulation of gas in the bag can cause the bags to float on the top of solid digesta within the rumen and therefore reduce digestion within it.

Many workers found close agreement and the same ranking order of degradability of protein sources from measurements made *in vivo* using abomasally canulated animals and using the bag method with correction for passage rate.

Mathers and Miller (1981) used four diets with varying proportions of lucerne and barley given to sheep at the maintenance level of feeding. The *in sacco* determination was made with the animals fed the corresponding diet. Using an assumed rumen passage rate of 0.046 (Ørskov and McDonald, 1979) the agreement between the *in sacco* and *in vivo* estimates was very good (see Table 2). Similar observations have been reported by Gonzalez *et al*. (1979).

Kennedy *et al*. (1982) in a study with sheep of the effect of environmental temperature on rumen passage rate, found that *in sacco* estimates tended to be lower than *in vivo* estimates. But, the ranking of feeds and effects of temperature on estimated degradability were similar (see Table 3).

Stern and Satter (1982) reported reasonable correlations between N disappearance *in sacco* at 12, 17 and 24 hr of 0.56, 0.55 and 0.68 respectively with *in vivo* protein degradability of 34 mixed rations determined with lactating cows.

TABLE 2

Comparison of estimates of the degradability of dietary protein determined in sheep

Method	Diet				S.E. ±
	Lucerne	2 Lucerne 1 Barley	2 Barley 1 Lucerne	Barley	
In vivo					
^{35}S incorporation	0.72	0.76	0.86	0.86	0.04
In sacco					
Ørskov and McDonald (1979) equation assuming $k = 0.046\ hr^{-1}$	0.73	0.72	0.78	0.86	0.01

*Mathers and Miller (1981)

TABLE 3

Comparison of estimates of the degradability of dietary protein determined in sheep

Diet / Environment	Barley Canolameal		Lucerne		Brome-grass Hay	
	Warm	Cold	Warm	Cold	Warm	Cold
In vivo						
^{35}S incorporation	0.90	0.89	0.80	0.76	0.60	0.51
passage rate $^{103}(Ru)$	0.026	0.031	0.054	0.081	0.064	0.065
In sacco						
Ørskov and McDonald (1979) equation	0.85	0.83	0.71	0.68	0.56	0.56

*Kennedy *et al.* (1982)

Meyer and Van der Walt (1983) studied three diets in sheep and also determined rumen passage rates with ^{103}Ru. Agreement and ranking between the methods was reasonable for two of the diets. However, correlation is required not just with traditional *in vivo* methods but with production trials in the field.

The advantage of the *in sacco* method lies in the ability to follow sequentially the rate of protein degradation. It also allows the use of animals fed on commercial rations.

A further advantage is the ability to study the nutritive value of feed residue after rumen incubation in order to estimate the qualitative contribution of undegradable protein to the duodenum (Mathers *et al*. (1979)

However, errors can arise from this method if inadequate consideration is given to the possible loss of the fine particles from the bag by washing. Also, soluble proteins are considered to be rapidly degraded but serum albumin and ovalbumin are examples of soluble but slowly degraded proteins (Annison, 1956; Mangan, 1972).

The technique suffers from standardisation problems, such as the selection of appropriate incubation time. Mathers *et al*. (1977) found an incubation of 4-6 hr gave the best prediction of *in vivo* degradability for the feed studied. While Ørskov and Mehrez (1977) suggested determining the extent of degradation when 90% of digestible dry matter had disappeared from the bag.

A third approach is to calculate a rate constant of disappearance from the logarithmic plot of the proportion of nitrogen remaining in the residue (Mohammed and Smith, 1977).

No details of comparison between laboratories have been published but Donaldson, Offer and Thompson (1981) reported considerable differences between Scottish Agricultural Colleges using a clearly standardised procedure and conclude that the value of the technique for routine feed evaluation is limited until the reasons for this variation can be identified and overcome. The technique also still requires the use of surgically modified animals.

The rate of degradation of protein in the rumen measured by this

technique may also be influenced by ruminal protease activity and pH (Ganev *et al.*, 1979) and there has been the suggestion of an increased rate or extent of degradation when micro-organisms have been allowed time to adapt to the particular test protein (Mohammed and Smith, 1977). If this proved to be correct, it would be a major drawback to the routine use of rapid *in sacco* or *in vitro* methods.

An additional problem is that the technique assumes that nitrogen remaining in the bags is undegraded dietary N, but using the ^{35}S infusion technique contamination with adhering bacteria can be shown to occur (Mathers and Aitchison, 1981). However, the error is only appreciable for materials which leave a substantial amount of low N residue in the bags.

Conclusions

The *in sacco* technique has been very useful in describing differences between feedstuffs, and is currently the best routine method for estimating degradability. There are still major problems of standardisation, reproducibility and interpretation to be resolved.

III - *In vitro* Laboratory Methods

In vitro technique is the laboratory method for feed evaluation which uses incubation of the feed with micro-organisms or semi-purified enzyme preparations or both (Ewart, 1974). *In vitro* estimation of protein degradability also includes measuring the solubility of protein either in water or in a saline solution.

(a) Solubility

The observed relationship between protein digestion and solubility in buffer suggested that protein solubility might be used as a simple method for ranking feeds in terms of degradability. Henderikx and Martin (1963), using purified proteins, reported a very high correlation (r = 0.99) between nitrogen solubility in a 10% mineral mixture (Burroughs *et al*. (1950) and *in vitro* degradation of proteins during a 6 hr incubation in rumen fluid.

Many different methods have been used for nitrogen extraction from feedstuffs (Annison, 1956; Crooker *et al*., 1978; Henderikx and Martin, 1963; Tagari *et al*., 1962; Wohlt *et al*., 1973). Different soluble nitrogen values have been obtained for similar feedstuffs depending on whether the solvent is water, autoclaved rumen fluid, mineral buffers based on the composition of saliva, rumen fluid or simple salt solutions. The nitrogen extracted from feedstuffs with salt solutions can be divided into:

1. non-protein nitrogen fraction (NPN);
2. protein nitrogen fraction.

The NPN fraction in feedstuffs is readily soluble. Solubility of the protein fraction is affected by several factors, including:

- degree of agitation
- length of extraction time
- temperature of extraction
- pH
- chemical composition
- ionic strength of solvent

} Lehninger (1970)

sample particle size	Wohlt *et al*. (1973)
heat treatment	Tagari *et al* (1962)
chemical treatment	Peter *et al*. (1971)
processing of feedstuffs	Wohlt *et al*. (1976)
CO_2 tension of the solvent	Wohlt *et al*. (1973)

Crooker *et al*. (1978), tested different solvents (Burroughs Mineral Mixture, McDougal's artificial saliva and sodium chloride) for nitrogen extraction from seven feedstuffs. They found no significant difference among the mean soluble nitrogen values from either Burrough's mineral mixture or sodium chloride. They suggested that these solvents may be used for the extraction of nitrogen from most feedstuffs.

Workers in the USA have been studying the possibility of formulating rations on such a solubility basis. Several workers have observed significant differences in milk production with cows fed on a protein solubility basis, and lactating dairy cows in these studies performed best on a ration with low solubility (Majdaub *et al*., 1978). However, the conditions used by Henderikx and Martin (1963) and others were such that all proteins were tested in the solution at very low concentrations, that even the most insoluble of proteins was actually in solution and the results indicate the protein in solution degrades at very different rates. This is a reflection of some other attributes of the protein, such as its structure, which also correlates with solubility index under defined conditions. However, solubility measurements describe only the fraction of total nitrogen immediately available for bacterial degradation, and will not describe the degradation of nitrogen not immediately soluble and which is degraded at a variety of characteristic rates. Therefore, solubility is not synonymous with degradability as has been assumed in many studies. For example, the solubilities of bovine albumin and or albumin are very high, but both proteins show strong resistance to hydrolysis by rumen micro-organism (Annison, 1956; Mangan, 1972). The reason for the resistance of these soluble proteins to degradation by rumen proteolytic enzymes has not been studied, but may be related to the presence of sulphur crosslinks.

Another example: the solubility of barley protein is quite low, values varying from 17-31% (Waldo and Georing, 1979 and Wohlt *et al*. 1973), but degradability values *in vivo* are 89% (Mathers and Miller 1977), and estimates from polyester bag studies range from 69 to 89% (Mathers and Miller, 1981; Mehrez and Ørskov, 1978).

Considerable variation in solubility in one solvent occurs between samples of nominally identical feedstuff. Waldo and Goering (1979) give the mean solubility of 101 samples of soya bean meal in 10% Burroughs *et al*. (1950) solution as 24.4% with standard deviation of ±7.73 units. The variation in solubility suggests there is a varying degree of heat treatment during processing of soya.

Similarly, the proportion of soluble N in fish meals is greatly influenced by the extent of autolysis of the raw material, and this in turn, depends on the species of the fish, time of storage before processing and whether or not preservative agents are added.

These studies indicate the possibility of developing equations relating solubility to degradability within one type of feedstuff.

Solubility measurements corrected by a fixed factor for each raw material, which is derived by a comparison of solubility at 6 hr *in vitro*, has been suggested as a routine procedure for protein evaluation of feedstuffs by the French (PDI) system (Institut National de la Recherche Agronomique, 1978; cited in Wilson and Strachan, 1981).

However, many of the above studies indicate that soluble protein is not necessarily equal to degradable protein, and that insoluble proteins are degraded at varying rates. Solubility measurements alone do not indicate degradability, and furthermore, they are unlikely to be used to predict both rate and extent of protein degradation.

(b) Incubation with rumen fluid

A very simple procedure is to incubate the feed sample with buffered rumen liquor for timed periods. Reviews concerning early developments with *in vitro* techniques have been written by Bentley (1959) and Johnson (1963). These reviews have presented a comprehensive

discussion of the historical development of *in vitro* rumen fermentation techniques and their uses for studying rumen microbial activity.

The *in vitro* digestion of protein by rumen micro-organisms involving natural feedstuffs as substrates has been reported earlier (Annison, 1956; McDonald, 1954). Frequently, increases in ruminal ammonia have been used as an index of proteolytic activity (Annison *et al*., 1954; Elshazly, 1952). However, the measured ammonia concentrations are greatly influenced by microbial utilisation and by the amount and nature of carbohydrate that is fermented (Crooker *et al*., 1978; Chamberlain and Thomas, 1979b). Using high starch concentrates the pH of the incubation mixture declines to low values and the apparent extent of protein degradation was reduced compared with the *in vitro* value (Laycock, Miller and Owens, 1980; cited in Miller and Laycock, 1983).

Another potential problem with the ammonia-amino acid method is that the accumulation of degradation end-products (which does not occur *in vivo*) may inhibit degradation.

Also, to obtain an effective degradability, it is necessary to correct degradation rates measured *in vitro* for passage rate.

Broderick (1978) developed an *in vitro* procedure for measuring ruminal protein degradation rate from end-products accumulation (ammonia + amino acid). Hydrazine sulphate was used to inhibit re-utilisation of amino acids and ammonia released by proteolysis with rumen fluid and the rate of degradation was determined from the increment in amino acid and ammonia concentration. Problems of maintenance of strict anaerobiosis and end-product inhibition of proteolysis were observed. To avoid the latter, incubation was carried out for only 2-3 hr. Comparison of the *in vitro* procedure, using hydrazine, in which degradation was measured either by accumulation of ammonia + amino acids or by measuring undegraded casein using alkali-labelled phosphorous (ALP) (see McDonald and Hall, 1957) as a natural marker bound to casein suggested that the former method was a more precise index of *in vitro* ruminal degradation. The ALP results also indicated that hydrazine sulphate was not inhibitory to protein degradation over 3 hr incubations.

Broderick and Balthrop (1979) studied the effect of various chemical

inhibitors of amino acid deamination using casein hydrolysate as a substrate in a batch-type ruminal _in vitro_ system. The results showed that hydrazine gives the highest recoveries of total amino acids and ammonia. Hydrazine inhibited microbial growth by inhibiting vitamin B_6, which may be potentially useful for reducing ruminal amino acid deamination. An extensive list of inhibitors is also given by Kitts and Underkofler (1954). The Broderick technique seems adequate for a soluble protein such as casein, but it requires modification before application to more complex feedstuffs containing proteins of varying solubilities and degradabilities, as otherwise only the more rapidly degradable components would be determined.

Recently, Broderick and Craig (1980) working with cottonseed meal protein noted that its degradation can be resolved into at least two separate first-order processes. The first fraction was degraded rapidly (0.68-1.19 hr^{-1}) which is more than 2-3 times faster than that of casein (0.34 hr^{-1}). Degradation rates of the second fraction were much slower (0.011-0.093 hr^{-1}).

Another recent approach is described by Mahadevan _et al_. (1979, 1980). The test protein is first converted into a brightly coloured diazo derivative by diazotisation with a marker molecule. It is then incubated _in vitro_ with rumen micro-organisms and subsequently the amount of undigested, acid precipitable diazo-protein is determined. Obviously, modification of the protein by diazotisation must not alter its susceptibility to degradation. The evidence supporting this assumption was limited and depended on the observation that diazotisation did not affect the trypsin-catalysed proteolysis of soya bean meal. Nugent (1979) showed that diazotisation altered the electrophoresis characteristics of serum albumin but that of casein was unaffected. Depending on the chemical structure of the protein, diazotisation may cause more or less change in structure with possible effects on the rate of proteolysis.

Another problem with this technique is that when diazo proteins are incubated with rumen fluid, the majority of the marker was removed rapidly within the first hour.

Cronje and Mackie (1983) compared the diazo method to the _in sacco_

technique, using soya bean meal, fish meal and maize gluten. Marked differences in binding of the coloured marker to the feedstuff protein were obtained. The estimates of degradabilities for fish meal and maize gluten were unrealistically high because of the rapid removal of the marker. Also they suggested that the marker attaches only to the fraction more rapidly degraded by micro-organisms and the technique could not indicate the rate or extent of degradation of the whole feedstuff (see Table 4).

Raab et al (1983), described a method where the ammonia was released from feedstuffs incubated in vitro. The incorporation of ammonia into microbial protein is calculated using the known relationship between fermentation (estimated from gas production) and microbial protein. Ammonia incorporation was calculated from linear regressions of NH_3 - N concentration (mg) and gas production (cm^3). The intercept (b_o) represented that amount of NH_3 - N which would be released when no fermentable carbohydrates were available and consequently no microbial protein synthesis took place (see Figure 3). The difference between this intercept (b_o) and the NH_3 - N content in the blank (rumen fluid without substrate added) indicated the amount of NH_3 liberated from protein and other N-containing compounds in the feeding stuff incubated.

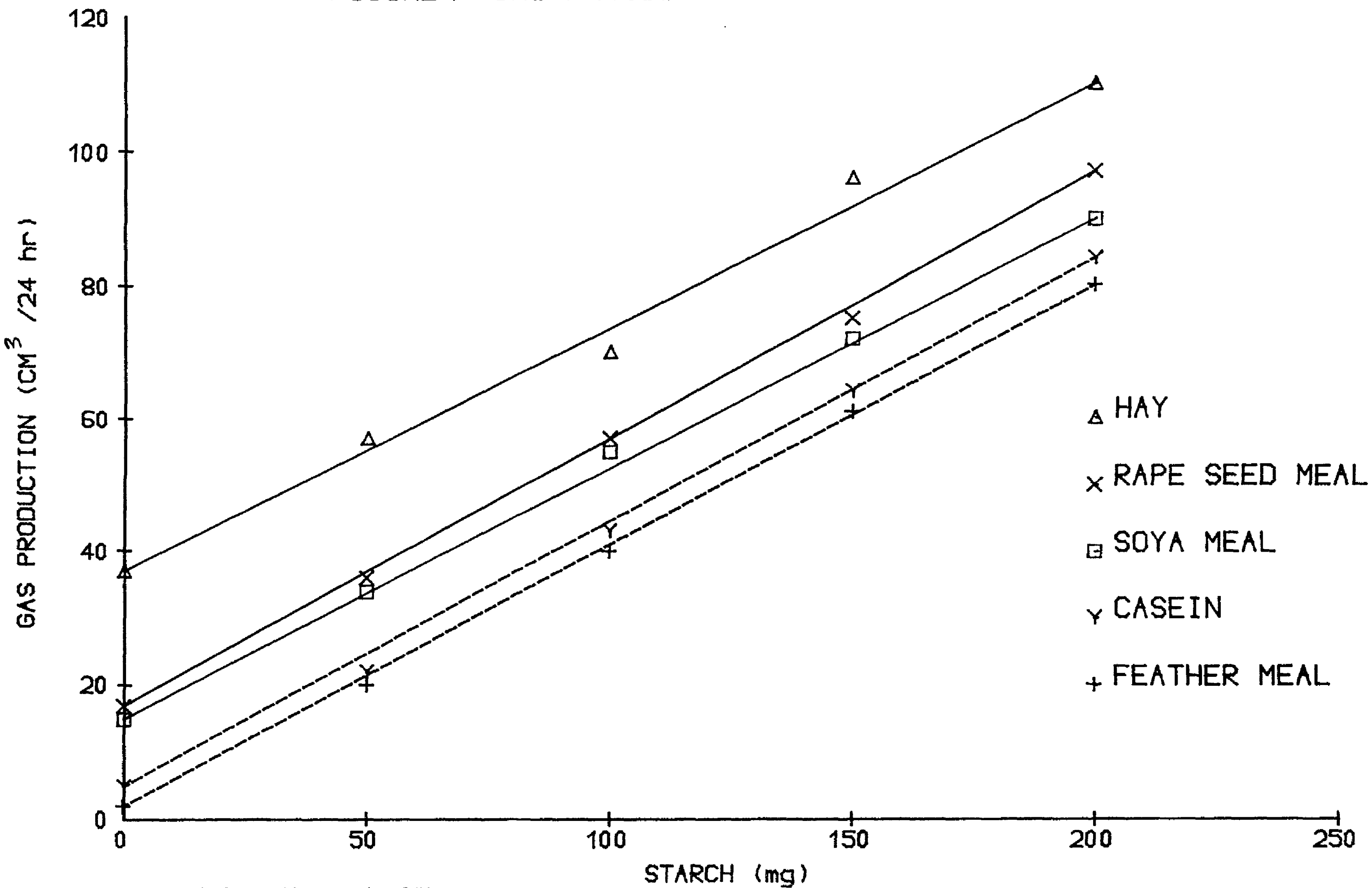
The in vitro degradable nitrogen (IVDN) was calculated as a proportion of the total N by the equation:

$$IVDN = \frac{NH_3\text{-N at zero gas production } (b_o) - NH_3\text{-N of blank}}{\text{total N of feeding stuff incubated}}$$

The period of incubation adopted was 24 hr, but different incubation periods can also be used. However, differences in fermentation patterns (VFA production) may cause different ATP:CO_2 production values, and these may be reflected in the regression lines between gas production and NH_3-N utilisation. Also, estimates of protein synthesis from NH_3-N disappearance and gas production ignore the utilisation of amino acids and peptides for microbial protein synthesis.

Another approach is described by Dennison and Phillips (1983). They measured the total amino acid content after 48 hr of incubation with an inoculum of rumen fluid. This is assumed to represent the sum

FIGURE 3: GAS PRODUCTION(CM^3/24 hr) IN VITRO



*RAAB ET AL, (1983)

T A B L E 4

N degradability estimated by loss of diazoprotein *in vitro* and by loss of N from polyester bags suspended in rumen of sheep

Incubation time (hr)	Loss of diazoprotein (%)			Loss of N *in sacco* (%)		
	Soya	Fish	Maize gluten	Soya	Fish	Maize gluten
1	89.9	69.4 (33.9)+	51.1 (8.9)+	64.8	25.8	13.5
6	95.9	78.3 (38.3)	32.5 (5.7)	91.2	30.0	17.0
12	95.9	80.6 (39.4)	63.2 (11.0)	97.3	35.0	21.0
Dye bound by 6.25 mg CP	0.659	0.322	0.115			

+Values in brackets are adjusted for relative dye bound by proteins compared with soya.

*Cronje and Mackie (1983)

of microbial amino acid, arising from microbial synthesis, and undegraded dietary amino acids from the feedstuff. The potential problems with this scheme were that no evidence was provided to show that the amino acid content after 48 hr in batch culture relates to the flow of amino acids to the duodenum of ruminants. Also the effect of the rumen on the energetic efficiency of microbial protein synthesis is ignored. Owens (1979) cited in Miller and Laycock (1983) reported a simple procedure in which the feed sample was incubated with buffered rumen liquor for a timed period. The mixture is then filtered on a Buchner funnel through the same polyester material as used in the bag technique and residual feed N is washed to remove bacteria. Disappearance curves for the protein sources by this procedure closely paralleled those obtained with bags in the rumen. However, differences between incubation of feeds *in sacco* within the rumen and the method described above may occur because degradation within bags is often delayed (Laycock and Miller, 1983). Also there may be differences in the case of high-starch diets, because the *in vitro* procedure is more likely to suffer from the effects of low pH.

(c) Incubation with proteolytic enzymes

Rumen inoculum is variable and requires the maintenance of donor animals. An alternative is to mimic the proteolytic activity of the rumen with purified enzymes. Unfortunately, the majority of proteolytic activity of rumen micro-organisms is bound in the cell wall so that separation of proteolytic activity has been difficult. Therefore, most studies have used commercial proteases derived from non-rumen sources.

1. Protease from *Bacteroides amylophilus*

B. amylophilus was shown by Abou Akkada and Blackburn (1963) to be one of the principal proteolytic organisms in the rumen. Further, they have shown that the *B. amylophilus* strain H18 does not have amino acid deaminease activity. Thus, a partially purified protease from this organism offers a distinct advantage in allowing determination of protein degradation by measurement of liberated amino

acids by the ninhydrin method since their usual rapid deamination is avoided. Mahadevan et al. (1980) reported similar results using soya bean meal. Their results indicated that soluble proteins from soya bean meal were degraded at similar rates. Further, degradation was the same using either the crude microbial fraction or the partially purified B.amylophilus enzyme (see Table 5). Among the pure soluble proteins, casein was most rapidly degraded by the protease and none of the three soluble protein preparations (serum albumin, soya meal and fish meal) was hydrolysed as rapidly as casein. These results show that soluble proteins may not all be degraded at the same rate by rumen proteases. However, a lag in hydrolysis of up to 6 hr was observed, probably due to the inaccessibility of the susceptible sites of the proteins to the protease. Therefore, most of the incubations were continued up to 18 hr. The reaction was linear between 6 and 18 hr, and rates of hydrolysis were calculated from the values obtained for the 12 hr interval between 6 hr and 18 hr incubations. No evidence has been produced to indicate that the system used gives values which correlate with in vivo or in sacco estimates of degradability.

2. Protease from Streptomyces griseus

Pickard and Van Soest (1977) were the first to propose the use of protease from S.griseus. This enzyme is active at pH 6.7-7.0, although the optimum is pH 8, and has a very broad specificity similar to that of mixed rumen micro-organisms. Degradation of protein sources is calculated by difference following measurement of the N recovered in the residue obtained by the filtration of the incubation mixture. The enzyme solubilises a very large part of the insoluble protein in forage (Pichard and Van Soest 1977) and in maize and soya (Krishnamoorthy, 1982). Rates of solubilisation of protein using this protease are very much more rapid than the rate of disappearance of N using the in sacco technique. Reducing the enzyme concentration to levels comparable to that of rumen fluid results in unphysiological rates of solubilisation of the test proteins. For the standard procedure incubation times of 18 hr for concentrates and 48 hr for roughages were adopted. The enzyme concentration was 3.3 units in 50 ml of pH 8.0 borate-phosphate buffer (Krishnamoorthy, 1982; Krishnamoorthy et al., 1982). The

TABLE 5

Proteolysis of soluble, insoluble and mercaptoethanol treated proteins with protease from *B.amylophilus* or crude rumen microbial fractions (μ moles amino acids/mg enzyme protein/hr)

Method	Feedstuff protein	Control	Mercapto-ethanol
B.amylophilus protease	Serum albumin (soluble)	933	2932
"	Rape seed meal (insoluble)	698	1212
"	Fish meal (insoluble)	657	2603
Crude microbial fraction	Diazo fish meal (insoluble)	6.2	57.7
B.amylophilus protease	Casein (soluble)	5333	-
"	Soya bean meal (soluble)	1365	-
"	Rape seed meal (soluble)	1860	-
"	Fish meal (soluble)	3250	-

*Mahadevan *et al*. (1980)

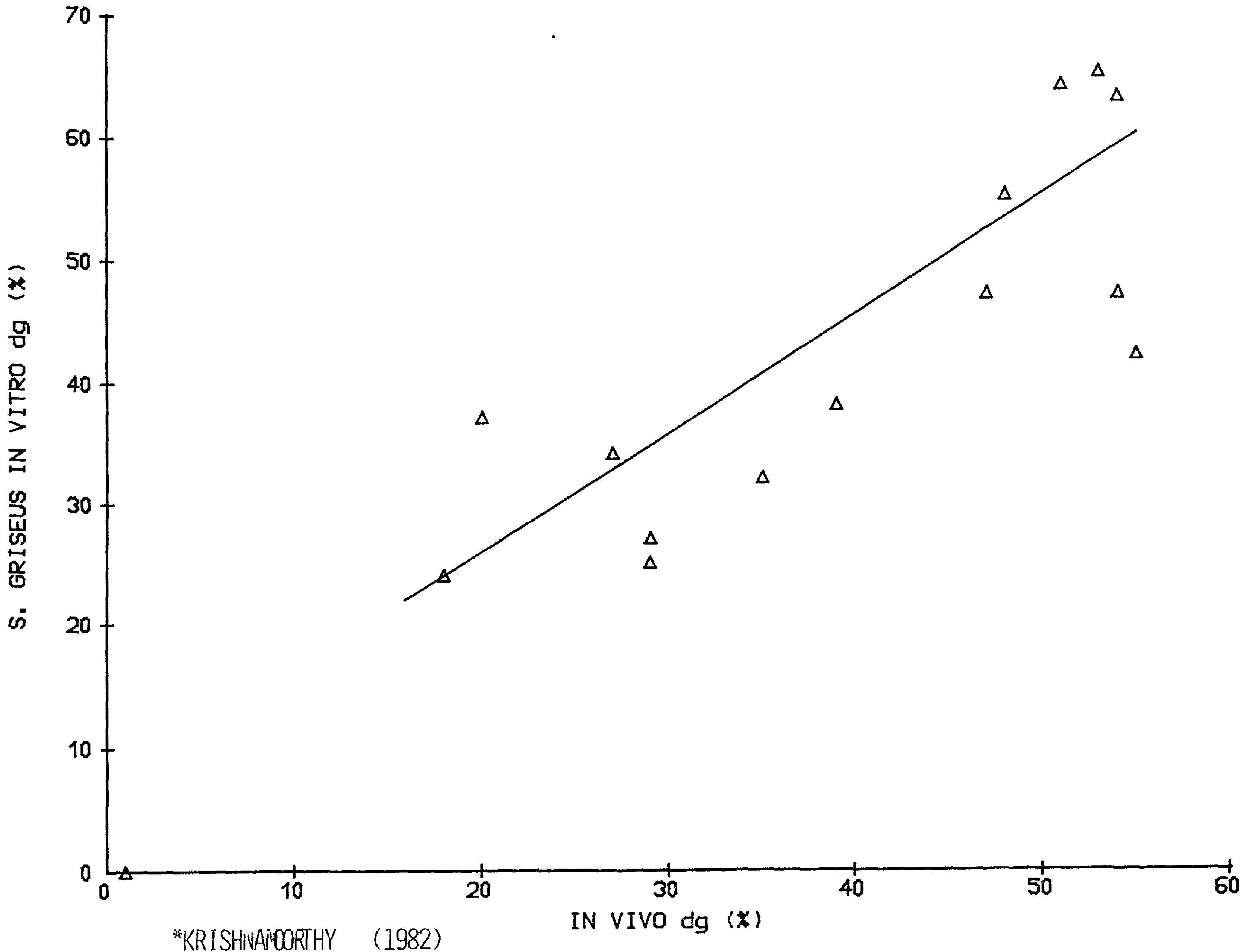


FIGURE 4: CORRELATION BETWEEN IN VITRO & IN VIVO DEGRADABILITY
S. GRISEUS IN VITRO dg (%)
IN VIVO dg (%)
0
10
20
30
40
50
60
70
*KRISHNAMOORTHY (1982)

choice of incubation times was based on the average mean retention time of concentrates and roughages in the rumen. The incubation time for roughages (48 hr) is relatively long. Krishnamoorthy (1982) applied this technique to the same feeds as used by Stern and Satter (1982) for *in vivo* measurements (see Figure 4). They found that enzyme degradability relates to the *in vivo* values according to the following equation:

predicted degradability% = 8.96 + 0.85 (*in vivo* degradability %)

Miller and Laycock (1983) examined this technique by separating soluble dietary protein from insoluble protein by filtration. They found that part of the soluble N passing through the filter following incubation with the enzyme is still precipitable by trichloro acetic acid (TCA), so that the enzyme does not degrade protein completely to small peptides and amino acids.

Very different results are obtained if the incubation is terminated with TCA. This may explain the relatively low degradation rates for groundnut meal and soya bean meal obtained by Chamberlain and Thomas (1979) using protease from *S.griseus*.

Krishnamoorthy (1982) reported rapid disappearance of N at early times of incubation using this technique compared with disappearance from bags *in sacco*. These differences may be attributed to differences in solubility of the feed protein in buffer compared to that in rumen fluid and to partial digestion of peptides using the *in vitro* technique.

Cottrill (1983) also examined the *S.griseus* protease method using feedstuffs available in the UK. The results are compared with estimates of degradability from dacron bag measurement where these were available. For concentrates and compound feeds the method produces similar ranking to dacron bag method at 18 hr incubation (Table 6). There is reasonable correlation with dacron bag estimates of degradability for protein concentrates, and the method proved capable of identifying proteins of low degradability (protected protein). For silage the protease method appears less sensitive to variation in apparent degradability. The method of preparing silages for analysis may affect the predicted degradability

using this method.

The protease technique is relatively easy to use and has the potential for a qualitative description of protein degradability. However, it does not always rank the protein in the same order as degradabilities estimated *in vivo* (Chamberlain and Thomas, 1979). The purified proteolytic enzymes may have different specificities for protein structures from thos of mixed rumen bacteria. Further work is required to modify the technique to provide estimates of degradation rates rather than single point estimates of degradability.

3. Natural fungal protease

Poos *et al*. (1980) reported the use of a neutral fungal protease. They compared this method with the dacron bag method to estimate protein degradability of samples of nine classes of feedstuffs. High correlations were obtained between N disappearance at 1, 4, 8 and 24 hr of incubation with the proportion of protein passing undegraded from the rumen as estimated by beef cattle performance trials.

Miller and Laycock (1983) compared this technique with the *in sacco* method and found that fungal protease method underestimates degradability. They showed that TCA soluble end-products were similar to those obtained with protease from *S. griseus*.

TABLE 6

Comparison of estimate of degradability of dietary protein determined by protease from S.griseus and by loss of N from dacron bag

Diet	% CP	Protease (18 hr)	Dacron bag (18 hr)
a. Concentrates			
Barley	8.6	31.4	71.5
Barley + Provimi 66	31.9	47.8	41.0
Ext. Rape seed meal	33.8	51.8	44.0
HCHO/Rape seed meal	34.8	9.2	11.5
Ext. Soya bean meal	43.0	80.4	72.1
Ext. Soya bean meal	44.0	82.1	76.4
Ext. Soya bean meal (C)	44.1	74.8	64.5
(HCHO)	44.5	9.4	15.0
Barley + Sopralin	31.5	8.3	15.0
Burgess Whole Soya	34.8	85.4	76.8
Burgess Extended Soya	37.8	65.1	50.0
Dried molassed beet pulp	7.6	85.2	76.7
Herring meal	66.8	50.8	44.6
Compound 1	15.9	45.1	47.0
Compound 2	14.6	67.1	72.0
Diet	**% CP**	**Protease (48 hr)**	**Dacron bag (48 hr)**
b. Forages			
Fresh grass	17.0	84.2	89.4
Grass hay (1003)	10.2	77.3	74.6
Grass hay (1004)	8.4	83.6	76.2
Grass hay	5.4	75.3	66.6
Grass silage (7784)	13.1	86.1	80.6
Grass silage (7785)	10.9	81.7	71.8
Grass silage (7786)	20.9	86.5	86.4
Grass silage (7787)	17.8	86.8	78.4
Grass silage - control	18.8	83.7	-
Grass silage + F100	15.9	85.9	-
Maize silage	8.2	81.7	64.3

*Cottrill (1983)

Conclusions

Solubility measurements are of little or no guide to degradability when applied to a range of feedstuffs but may be of use when considering effects of processing on variation within a specific class of feed. The measurement of ammonia and amino nitrogen release on incubation with rumen inoculum, appeared to be attractive for handling large numbers of samples, since it cheap and easy to use. However, the method suffers from poor replication due mainly to variation in microbial assimilation of ammonia resulting from differences in energy content of feeds.

One promising method appears to overcome this limitation by incubating substrates in a well controlled artificial rumen fermenter (Rusilec technique) developed by Czerkawski and Breckenridge (1977), where the rumen condition appears to be maintained for several months. Such fermenters could be very useful if they can be adequately controlled in laboratories with no animals. However, this method needs a comparatively complex procedure.

The protease techniques (fungal or bacterial) are easy, straightforward, and give a qualitative description of protein degradability. Although rate constants can be calculated, they do not always rank the protein in the same order as degradabilities *in vivo*. Also, they fail to mimic the *in sacco* results, this may be due to the basic nature of the enzyme or could be a simple problem concerning enzyme to substrate concentrations. In all *in vitro* methods it is essential that degradation is described in relation to time, otherwise the measurements are of very limited value since they cannot be adjusted adequately for passage rate. A single estimate of degradation is therefore inadequate. This is particularly important since the ranking order of different protein supplements changes, or their relative differences change with different passage rates.

SECTION 2

RATE OF PASSAGE OF DIGESTA THROUGH THE RUMINANT DIGESTIVE TRACT

Introduction

Different terms have been used to describe the passage rate of material through the digestive tract including, transit time, retention time, passage rate, rate of flow and rate of transport. The 'passage rate' has come to mean the quantity of digesta (as weight or proportion) that passes a point along the alimentary tract in a given time (Balch and Campling, 1965). From a nutritional viewpoint the passage rate is one of the important factors that determines the efficiency of feed utilisation; others include the rate of digestion, the nature of the absorbed end products of digestion and the requirements of the animal.

The passage rate through the ruminant digestive tract is influenced by the following factors.

(a) Feed intake and level of feeding (Bines and Davey, 1970; Freer and Campling, 1963; Grovum and Williams, 1977; Owens *et al*., 1979).

(b) Physical form of the diet (Coombe *et al*., 1979)

(c) Dietary roughage level (Cole *et al*., 1976; Owens *et al*., 1979; Prange *et al*., 1979).

(d) Changes in the rate of break down (Balch and Crampling, 1965).

(e) Specific gravity of digesta particles (Balch and Campling, 1965).

(f) Stage of pregnancy (Graham and Williams, 1962).

The contents of the rumen can be visualsed as consisting of a liquid phase and a solid phase. The liquid fraction contains water, soluble feed components and nutrients solubilised by the degradative

processes of the micro-organisms. The solid fraction contains undegraded and indigestible material together with microbes adhering to the solids. Because the volume of water entering the rumen via animal ingestion is generally greater than the volume of solid material, and because further liquid is added from salivary secretions during mastication, liquid may leave the rumen at a rate faster than that exhibited by the solid fraction. Thus, the rate of removal of soluble nutrients from the rumen due to passage through the digestive tract would be expected to be greater than the rate of removal of insoluble undigested nutrients.

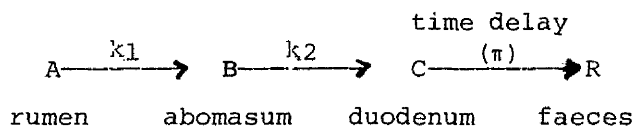
Isaacson et al. (1975) demonstrated that the amount of microbial protein synthesised per unit of carbohydrate fermented by rumen micro-organisms and the proportion of dietary undegraded protein are both increased with elevations in ruminal passage rate. A proposed mechanism for the increased efficiency of microbial protein synthesis was that the microbial maintenance requirement was reduced at higher ruminal passage rates.

Models used to caluclate rate of passage

Several models have been used to calculate the passage rates through the digestive tract. Short critiques of the more commonly used models are given:

Balch (1950) used the strained particle technique with cows and suggested the term '5% excretion time' as a measure of the time required for residues to traverse the omasum, abomasum and intestines. The interval of time between excretion of 5 and 80% of the particles was claimed to be indicative of the time taken for passage through the reticulo-rumen. These excretion times were calculated from the cumulative excretion curve.

Blaxter et al. (1956) suggested that the passage of ingesta through the digestive tract of ruminants be regarded as a kinetic process. They proposed a model system as follows:



where A, B, C and R represent fraction units of feed in the compartment concerned. Results obtained by the stained particle technique fitted a simple kinetic equation with the rate constants k_1 and k_2 and the time delay (π) between C and R. Manipulation of the equation permitted estimation of diurnal variation in faeces production. However, there is little experimental evidence as to the organs of the digestive tract to which these constants apply.

Brandt and Thacker (1958) indicated that the order of flow for two sequential compartments with first-order kinetics cannot be determined mathematically from excretion data. They described a model based on an analogy of a hydraulic flow through two volumes in which complete mixing takes place. They showed that the passage of digesta is an exponential function and that a single volume model with a single 'half time' was sufficient to describe the flow of feed residues in rabbits. For ruminants a two-volume model with two half-times was proposed. Their equation describing the ruminant model is similar to that developed by Blaxter _et al_. (1956)

Another model was suggested by Hungate (1966). He proposed a two-pool model for ruminants where coarse particles in the pool to be ruminated have not yet entered the pool of material leaving the rumen, but constitute a separate pool which feeds it. The average rate at which small particules leave this rumination pool is proportional to the concentration of coarse particles in the pool, and in consequence the passage from the coarse to the small particles pool follows the kinetics of a first-order reaction. This precedes the passage of the comminuted particles from the pool of rumen liquid and small particles to the omasum. Total passage of initially large particles from the rumen is thus the result of two sequential first order reactions.

To calculate 'turnover time' by Hungate's equation, the fraction of the original marker retained in the animal is plotted against the time after consumption of the marked feed. The turnover time is then calculated from the times taken for certain percentages of the marker dose to appear in the faeces.

TABLE 7

Classification of markers

External Markers	Internal Markers
particulates	silica
dyes	chromogen
metal oxides	acid soluble faecal fraction
water-soluble marker	methoxyl and fibre
radio active markers	
inert metals	
micro organisms	

Application of markers

The variety of information provided by markers in nutritional studies includes:

a. Food intake

b. Extent of passage

c. Passage of ingesta

d. Digestibility studies

e. Balance studies

f. Consumption of forage in grazing animals

g. Measurements of gut volume.

Grovum and Williams (1973a, c) developed a mathematical model of digesta passage through the alimentary tract of sheep. They showed that the passage rate of digesta could be described by an equation having as independent variables: the transit time of marker through the intestinal tract, the half-time of marker in the reticulo-rumen, and the half-time of marker pertaining mainly to the caecum and proximal colon. Their research indicates that the longer half-life is associated with reticulo-rumen passage and shorter half-life is associated with intestinal passage.

The same workers (Grovum and Williams, 1977) evaluated the above model by relating the measured values for passage rate to the apparent digestibility of the feed, the concentrations of volatile fatty acids (VFA) in the reticulo-rumen and the water content of the faeces.

Measurement of passage rate

In order to measure passage rate of the total feed intake it is necessary to identify residues from individual meals. This has been achieved by introducing a marker in the meal and then taking samples of digesta or faeces and recording the marker recovery. The marker should be non-toxic both to the animal and to its microbes; it should not be adsorbed across the rumen wall nor metabolised and should mix completely with the digesta throughout the gut. Adsorption, absorption and metabolism would lead to an overestimate of the dilution rate of the marker. Complete recovery and ease of measurement have been the characteristic of major concern in the search for ideal markers.

A nutritional marker, depending on the nature of both the marker and the study, may be administered with the feed after mixing with part or all of the diet, or drink, may be taken in a pill or capsule when its taste is an undesirable one. When it cannot be chewed it may be prepared as a powder or solution to be taken orally or through the fistula. Internal markers used in digestibility trials are ingested in measurable quantity as part of the diet.

A classification of markers used for feed utilisation or rate of feed passage is given in Table 7.

Requirements for markers used in passage rate measurements in the digestive tract are more critical than those for balance trials. Absolute measurements of passage rate for particles and liquid require recoverable markers which do not separate from their respective labelled fractions. The assumption is that the marker is at all times in equilibrium with the pool of the fraction that is labels. Also, the practical needs of passage studies make it desirable to have measurements of both liquid and particulate fractions of the digesta, since these are known to undergo relatively independent turnover.

A number of markers have been used to measure the passage rate of ingesta through the gastro-intestinal tract of ruminants:

1. Particulates

Brazil nut, charcoal, rubber pieces, glass beads, cotton , seeds, ball bearings and plastic pieces have been used in the past (Kotb and Luckey, 1972). While these markers yield information on relative passages, they are not amenable to the estimation of the absolute rates of digesta fractions required for modelling studies. In addition to the question of whether they move with the fraction they are intended to label, they are not easily quantifiable.

2. Dyes

Dyes have been used to stain feed since about 1920 (Kotb and Luckey, 1972). The stained particle technique was used by German and Finish investigators and subsequently by Balch (1950). However, this technique is laborious and time-consuming. Further, serious questions might be raised concerning the significance which can be attached to such flow measurements in view of the extra-active nature of the staining procedure and the selectivity involved in detecting such stained residues in faeces (Ellis and Huston, 1967).

3. Water-soluble markers

The most widely used water soluble marker is polyethylene glycol (PEG) which was introduced by Hyden (1955). He found that the PEG polymer with a mean molecular weight of 4000 was not degraded in the gut or absorbed, and described the use of PEG as a marker to estimate the

rate of passage of digesta, measurement of the gut volume and the rate of absorption of solutes from the gut. However, PEG has been shown to be excluded from a large proportion of the water in beet pulp (Czerkawski and Breckenbridge, 1969) and is precipitated when given feeds rich in tannins (Kay, 1969). Some PEG may be absorbed since there is recovery of only 95% in digesta and faeces of sheep (Downes and McDonald, 1964).

4. Chromic oxide

Chromium sequioxide (Cr_2O_3, M.W. 152.02) is a convenient solid passage marker. It is one of several chromium compounds with characteristics of inert indicators such- chromium chloride ($^{51}CrCl_3$) sodium dicromate ($Na_2{}^{51}CrO4$) and haemoglobin. Of all those, only Cr_2O_3 has been used widely in both radio-active and non radio-active forms in studies of digesta movement. The use of Cr_2O_3 as a faecal marker was first proposed by Edin (1918). A number of studies with sheep have shown that Cr_2O_3 given in the food or intraruminally passes through the stomach and intestines unabsorbed and can be recovered quantitatively in the faeces (MacRae, 1974). Kane et al. (1950) using a baked mixture of Cr_2O_3 powder and flour, obtained excellent recoveries (99%) in mature cows. Langland et al. (1963) with growing steers, found a mean recovery of approximately 94% with Cr_2O_3 powder mixed in the diet. In experiments with sheep using Cr_2O_3-paper, Thomas et al. (1980) obtained a mean recovery of 100%.

Among many solid markers Cr_2O_3 can be excluded as a good passage marker since its physical properties has little correspondence to those of the solid digestion fraction. However, the use of Cr_2O_3-powder or paper as indigestible markers was associated with variable faecal recoveries of Cr_2O_3 apparently because of variable degrees of retention of both markers in the rumen (Field, 1964; Chamberlain and Thomas, 1983).

Chamberlain and Thomas (1983) suggested the use of Cr_2O_3-impregnated straw as a marker because the indigestible nature of the straw ensures that the marker leaves the rumen still attached to the straw particles.

5. Micro-organisms

The passage of unattached bacteria in the sheep rumen was successfully monitored using alumina with traces of ^{192}Ir having a specific gravity similar to the bacteria (Bullen _et al._, 1953). It is unlikely that this method could be applied to normal rumen bacteria which may have attached to particulate matter with a consequently slower passage.

6. Radio-isotopes

Many radio-isotope markers have been used in studies of feed utilisation and rate of passage $^{51}CrCl_3$, $^{51}CrO_4$ and ^{51}Cr-EDTA. Of all those chromium-ethylene diamine tetra acetic acid (^{51}Cr-EDTA) has been used commonly in rate of passage measurements. It was proposed as a liquid marker by Downes and McDonald (1964). They concluded that ^{51}Cr-EDTA is a satisfactory soluble marker in spite of slight absorption and subsequent excretion in the urine and that the estimation of ^{51}Cr is simple, accurate and specific.

Where the use of radio-active indication is undesirable, the stable Cr-EDTA complex can be used. Binnerts _et al._ (1968) studied the stable complex in digestion experiments with ruminants and described a method for estimation by atomic absorption. Their results agreed with the results obtained by Downes and McDonald (1964) for the radio-active indicator. However, Dobson _et al._ (1976) found that the absorption was affected by osmotic pressure in the rumen and overestimation of the inflow and outflow of ruminal water of perhaps 10% could occur.

The radio isotope measuring methods are relatively easy to carry out with precision; the amount of radio-active indicator to be used is small and has no effect on the bulk of the feed and no undesirable side effect, and it is possible to obtain a detailed picture of the passage of material through the digestive tract.

7. Rare earths

Ellis (1968) has discussed a number of properties of the rare earth elements which suggested their advantages as indigestible markers. Some of those elements (such as Yttrium, dysprosium and cerium), in

addition to being indigestible by mammals are tightly bound to plant material and, therefore, might be expected to pass through the digestive tract in close association with indigestible feed residues. The adsorption of low concentrations of certain metals (mostly ^{144}Ce - ^{144}Pr complex) to feed particles has been used (Ellis and Huston, 1968). The adsorption occurs at concentrations approximately equal to the molar solubility of the corresponding hydroxide (10^{-7} - 10^{-6}M) for most rare earths and is possibly due to a cation exchange effect (Ellis and Huston, 1968). However, there is some evidence for adsorption onto rumen contents by dysprosium at concentrations of 10^{-5}M (Ellis, 1968). The rare earths offer a major advantage of ease of application without extracting soluble plant material and thereby influencing rate of fermentation and as a consequence, rate of passage.

8. Chromium-mordants

A technique for mordanting Cr and Ce to plant fibre, developed by Vas Soest, was used by Martz *et al*. (1974) where Cr-, and Ce- and Au-mordanted plant cell walls were incubated for 48 hr *in vitro* and then treated with neutral detergent. Recovery for Ce was 0-67% for Au 18-55% and for Cr 66-103%. The lowest Cr-recovery was associated with the highest *in vitro* cell wall digestibility (IVCWD) and the lowest amount of Cr on the fibre.

Rate of passage of protein supplememts from the rumen

Rate of passage studies indicate that small particles (e.g. protein supplements) pass from the rumen at rates intermediate to those of the liquid phase and the long roughage. Large particles remain longer in the rumen because of the time needed to reduce the particle size below the critical minimal size that can pass from the rumen. Mean retention time in the rumen of roughage depends mainly on the rate of fibre digestion as well as on the factors which influence rumen liquid passage rate such as variation in feed intake, roughage to concentrate ratio particle size of feed and environmental temperature.

There are few direct estimates of passage rate of feedstuff proteins from the rumen of cows and sheep because they are difficult to

obtain. However, it has been made easier since Ganev et al. (1979) have developed a technique to treat the protein supplement with sodium dichromate which renders the protein completely undegraded. The rate of passage of particles from the rumen can be determined simply by measuring the dilution rate of chromium from the rumen or faeces. The small effects of Cr-treatment on the particle size distribution and on density suggested that Cr-treated protein supplements are likely to behave in a manner similar to the original protein supplement. Further, it is assumed that small protein particles may leave the rumen without further degradation and hence the effect of Cr-treatment on the degradability of protein supplements is of less importance. The preliminary indication from the chromium studies by Ganev et al. (1979) indicated that the following passage rates for small particles may be representative of different situations:

Cattle and sheep given ground diets without roughage	$k = 0.02\ hr^{-1}$
Low yielding dairy cows, cattle and sheep on mixed diets	$k = 0.05\ hr^{-1}$
High yielding dairy cows	$k = 0.08\ hr^{-1}$

Using a similar technique Elimam and Ørskov (1982a) measured the passage rates of protein supplements (fish meal and soya bean meal) in sheep fed chopped dried grass at four levels of intake (see Table 8). They found that the passage rate of both protein supplement increased with the increase in feed intake. They observed no significant differences between supplements. Warners (1981) also observed a high degree of variation between animals.

Eliman and Ørskov (1982b) also measured the passage rates for Cr-treated fish meal particles in dairy cows given a mixed diet of hay and concentrate. They found that the passage rate increased with feeding level up to 2.5X maintenance and then levelled out (see Table 9). The overall relationship between feeding level and mean passage rate from the rumen was linear ($P < 0.01$). The effect of feeding level on passage rate was similar to that observed in sheep (Elimam and Ørskov, 1981a). However, the passage rates were

TABLE 8

Mean passage rates (hr^{-1}) of Cr-treated protein supplements from the rumen of sheep at different levels of feeding

Feeding level x maintenance requirements	Mean passage rate of Cr (hr^{-1})		Combined
	White fish meal	Soya bean meal	
0.5	0.010	0.010	0.010
1.0	0.019	0.026	0.022
1.5	0.032	0.034	0.033
2.0	0.038	0.039	0.039
SE	0.0032	0.0032	0.0023

*Elimam and Ørskov (1982a)

TABLE 9

Mean passage rates (hr^{-1}) of Cr-treated fish meal from the rumen of dairy cows at different levels of feeding

Feeding level (x maintenance requirements)	Mean passage rate of Cr (hr^{-1})
1.5	0.065
2.0	0.072
2.5	0.091
3.0	0.088
SE	0.005

*Elimam and Ørskov (1982b)

considerably greater with cattle due to the relatively higher feeding levels achieved in the dairy cows and their body size. The effect of feeding level observed by Elimam and Ørskov (1982 a, b) has been confirmed by Ganev et al. (1979); Blaxter et al. (1956) and Grovum and Williams (1977). The reason for the levelling off of passage rate at high feeding levels is not clear, but suggests there is a physiological limitation on passage rate.

The chromium mordant method used to study the rumen passage rate of small particles of protein supplements cannot be used for roughage or large particles since fibre digestion is also totally inhibited which greatly affects their passage rate. An alternative method is the use of rare earth markers to label small or large particles. Hartnell and Satter (1979b) determined rumen passage rate of a concentrate diet of 0.040 hr^{-1} for dry cows consuming 10.8 kg dry matter d^{-1} and 0.046 hr^{-1} for cows in mid lactation consuming 19.8 kg dry matter d^{-1}. The corresponding values for hay with rare earch markers were 0.034 hr^{-1} and 0.035 hr^{-1}. Meissner et al. (1979) reported values of 0.027 and 0.015 hr^{-1} respectively for cattle fed ad libitum coarse ground hay or straw and significantly greater values of 0.045 and 0.027 hr^{-1} for sheep offered the same diets. However, the passage rates for long roughage quoted above cannot be assumed to represent the retention time of the feed in the rumen, because these rates appear to represent the passage rate of small particles derived from the breakdown of long roughage. The results of Hartnell and Satter (1979b) suggested a greater retention time for hay compared to concentrate. During the additional retention time the microbes break down the long particle fraction to smaller particles thus allowing them to pass from the rumen.

McDonald (cited in ARC, 1983) suggested that it may be possible, when more information is available, to refine the concept developed for degradability and passage rate of small particles (Ørskov and Mcdonald, 1979). There is a need to establish k_1 which is the passage rate of small particles as before but in addition k_2, the rate of degradation of large to small particles. The effective degradability can then be expressed by the formula:

$$P = a + \frac{bc\ (C + k_1 + k_2)}{(C + k_1)\ (C + k_2)}$$

where a, b and c are the constants described on page 19.

The rate of reduction of large to small particles is also a key parameter in estimating voluntary intake in ruminants (Meissner *et al.*, 1979).

Effect of passage rate on protein degradation in the rumen

The degradability of dietary protein in the rumen constitutes an important part of the new protein system for assessing protein requirements for ruminants (ARC, 1980; 1983). The system suggested that feedstuffs did not have a constant degradability value but that value would vary with the nature of the basal diet and level of feeding. The passage rate of protein from the rumen also affects greatly the extent of degradation in the rumen. Consequently, it is important to have information on the passage rate of different protein sources from the rumen to determine the effective degradability of protein.

In many of the techniques used to measure protein degradability (e.g. bag techniques or laboratory techniques) the rate of microbial degradation of protein was determined in isolation from the passage rate of the protein from the rumen. If the passage rate is also determined, the effective degradation of dietary protein *in vivo* may be calculated from the amount of feed remaining at any time and the rate of passage of that material. Equations for this calculation have been described by Ørskov and McDonald (1979), Miller (1980) and McDonald (1981) (see page 19).

According to Ørskov and McDonald (1979) the effective degradability (P) can be calculated for the equation:

$$P = a + \frac{b\,c}{c + k}$$

where a, b, and c are the constants that describe the loss of the feed N from *in sacco* incubation ($P = a + b(1 - e^{-ct})$) at time t and k is the passage rate. With this dynamic approach, there can be no single value for the degradability of the protein in a particular feedstuff since it depends on the rumen passage rate.

Table 10 gives degradability values for the common source of protein supplements concentrate at three values of k (passage rate) which represent conditions likely to be found in practice (Ørskov, 1982). For protein supplements with a substantial a value but with little or no b value, then rumen passage rate will have little effect on actual degradability. For example with freshly processed fish meal calculated degradability decreases only from 22.7 to 21.5% when k increases from 0.02 to 0.08 hr^{-1} (see Table 10). The greatest effect of passage rate occurs with protein having a large b fraction and a low value for c. An example is linseed meal where degradability changes from 78.1 to 46.0% over the range of k from 0.02 to 0.08. Figure 5 illustrates that the differences in degradability between protein supplements are generally smaller at high passage rates. The adjustment of degradability for rumen passage rate sometimes also has the effect of changing the ranking order of protein sources so that a listing of degradability values at one particular passage rate can be misleading. In other words, some protein supplements are more suitable in feeding conditions giving low passage rate, and others are more suitable at high passage rate. For instance, Table 10 shows that meat and bone meal at passage rate of 0.08 is very similar (41.2) to linseed meal (46.0), while at the low passage rate of 0.02 the values are quite different (52.1 compared to 78.1). Similar results were obtained by Miller and Laycock (1983) (see Table 11).

Conclusions

Rates of protein degradation measured either *in sacco* or *in vitro* must be corrected for rumen passage rate to provide estimates of effective degradability for rationing purposes. For most vegetable proteins, fed at low levels of feeding and consequently low passage rate, very little protein will escape degradation in the rumen. Passage rate will not greatly affect protected proteins or proteins that are naturally resistant to rumen degradation. For high yielding cows with higher intakes and higher passage rate the differences in degradability between different feedstuff proteins are small.

More research is required to develop reliable techniques for determining passage rates of roughage particles. Also, there is a need for more understanding of the factors affecting passage rate, especially the large animal variation.

TABLE 10

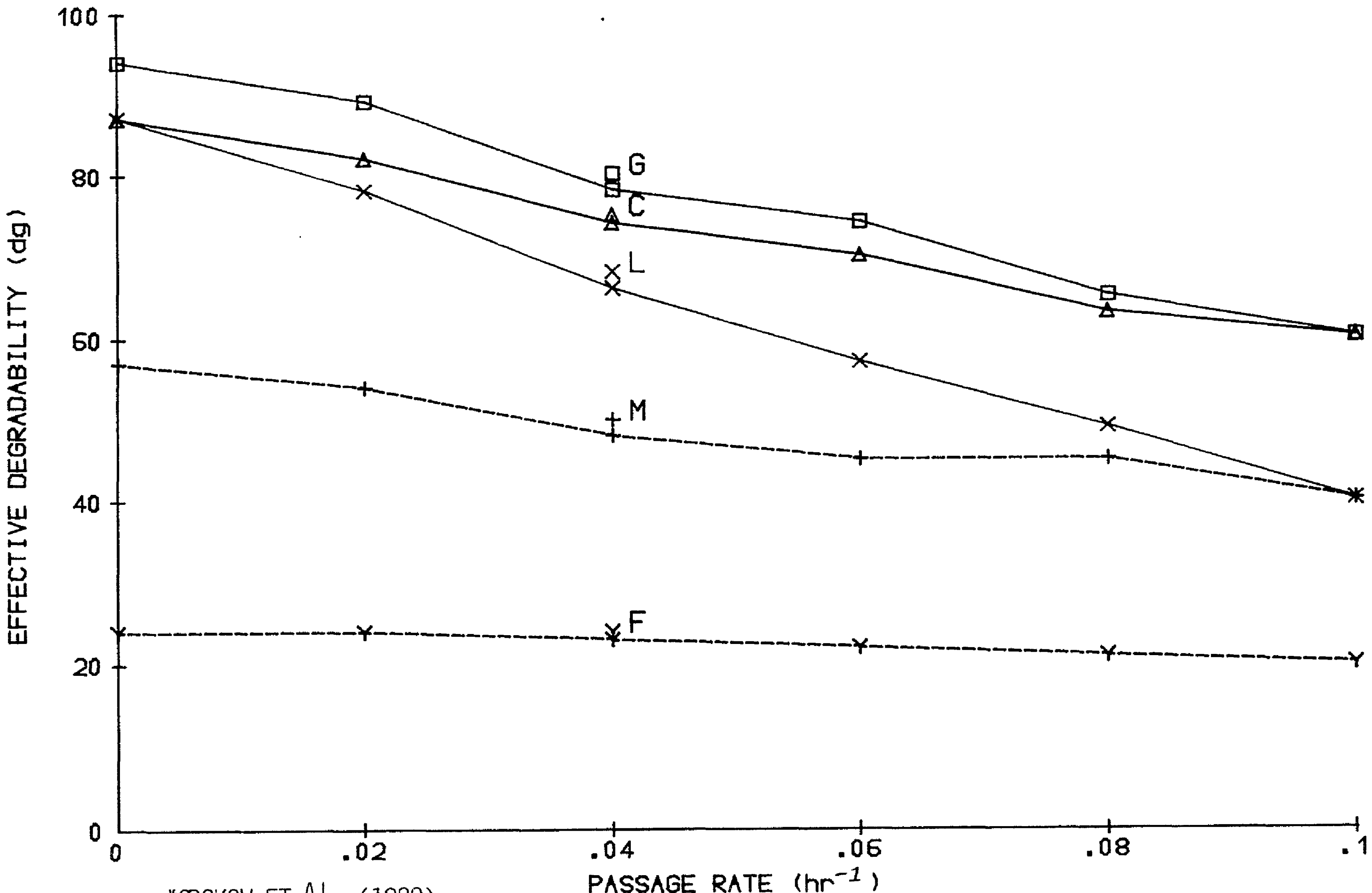
Degradability of several protein supplements at different passage rates[+]

Protein source	Degradability (%) at different passage rates (k)		
	0.02	0.05	0.08
White fish meal (unknown origin)	64.3	49.6	41.5
White fish meal (unknown origin)	72.6	58.5	52.4
Fishmeal stale at processing	61.7	51.6	47.7
Fishmeal freshly processed	22.7	22.0	21.5
Processed and preserved fish press cake	18.0	13.9	11.3
Meat and bone meal	52.1	45.4	41.2
Cottonseed	80.6	69.6	62.7
Linseed	78.1	58.9	46.0
Soyabean meal	80.8	62.5	50.4
Ground-nut meal	87.4	74.1	64.3
Sunflower meal	85.9	76.9	70.4
Guar meal	82.5	66.7	56.3
Sugar beet pulp	63.5	50.3	45.3
Ground peas	89.4	80.0	74.4
Beans (*Vicia faba*)	82.9	66.6	56.2
Spring rape	86.5	78.1	72.2
Autumn rape	88.7	78.6	71.5
Turnip rape	90.2	79.9	73.0

*Ørskov (1982)

+Degradation rates determined with roughage-fed animals

FIGURE 5: EFFECT OF DIFFERENT PASSAGE RATE ON DEGRADABILITY



*ØRSKOV ET AL., (1980)

TABLE 11

Effective protein degradability of three feedstuffs at various rumen passage rates, together with the relative ranking of the feedstuffs

Objective	Rumen passage rate (hr^{-1})		
	0.02	0.05	0.08
Degradability (%)			
Field beans	96	92	89
Soya bean meal	87	74	65
Fish meal	41	38	36
Ranking			
Field beans	2.37	2.45	2.50
Soya bean meal	2.14	1.96	1.83
Fish meal	1.00	1.00	1.00

*Miller and Laycock (1983)

SECTION 3

PROTEIN SUPPLEMENTATION OF DIETS FOR GROWING CATTLE

Introduction

Extensive work has been carried out looking at the effects of supplementing different diets with protein sources such as urea, soya bean meal, ground nut meal, oil seed cakes, beans, dried blood, meat and bone meal, and fish meal. Many of these supplements have been shown to improve various criteria of production; e.g. live weight gain, intake, feed conversion efficiency or nitrogen retention.

The majority of the work has been carried out with rations comprising forage and concentrate components, with the supplement being incorporated into the concentrate (Chalmers et al., 1982; Huber and Cook, 1972). Few trials have used complete diet feeding (Waterhouse et al., 1983; Ørskov et al., 1971).

Mode of action of protein supplements

According to Miller, 1978; Roy, 1980 and ACR,1980, growing cattle at the young ruminant stage have a requirement for UDP. As the animal matures the requirement for UDP decreases, ultimately becoming zero in the weight range 200-300 kg.

At the early stage of growth the tissue amino acid requirement can not be met by rumen microbial protein alone because the energy available to the microbes at this stage does not allow sufficient synthesis. Therefore, dietary UDP supplementation is necessary for the animal to receive the correct amino acid supply in the protein reaching the small intestine.

Thus, only young growing cattle should theoretically show a beneficial response to additional UDP provided the microbial RDP is met. Where energy, N and sulphur are not limiting, microbial protein produced in the rumen should be adequate to sustain maximum growth rates in growing cattle over 200 kg live weight. However, recent work with beef cattle over 200 kg live weight fed RDP adequate diets, has demonstrated that a response in intake or live weight gain to supplementary protein can be obtained (Forbes and Irwin, 1970; Kirby and Chalmers, 1981

Reasons for these responses may include one (or more) of the following factors:

1. Increase in the diet digestibility due possibly to the supply of microbial growth factors (Thomas *et al*., 1980; Bax and Offer 1982).

2. Increase in dry matter intake possibly facilitated by increased intestinal amino acid intake (Forbes and Irwin, 1970).

3. Increase in microbial protein yield due to the slow NH_3-N release from supplements in the rumen (Smith *et al*., 1982).

4. Increase in the UDP supply (Smith *et al*., 1982; Beever *et al*., 1982).

5. Increase in the supply of essential amino acids to the small intestine (Roy, 1980; Hennessy *et al*., 1981; Barry *et al*., 1982; Veen and Vahl, 1984).

These unexpected responses to protein supplementation in older growing cattle are still not fully understood as many of these studies have been done under different conditions. More detailed work is needed before the problems can be resolved.

Animal responses to protein supplementation (fish meal and urea)

Fish meal and urea were used as positive and negative control protein sources in the feeding trial to evaluate untreated and treated fish silages as supplements for the growing ruminant calf. To allow valid comparisons it is necessary firstly to review previous trials with these two protein sources. Previous work with fish silage has been reviewed in the following section.

(a) Fish meal

Fish meal is a good source of protein, essential amino acids (particularly lysine and sulphur amino acids), energy, minerals (particularly phosphorous), vitamins and essential fatty acids.

Its dry matter usually contains over 70% crude protein, while the remainder consists mainly of ash (10-20%) and fat (3-13%). Depending

on its origin and the way it is processed, different sources of fish meal may differ in their chemical and physical properties.

A considerable number of research papers conclude that fish meals contain unknown growth factors (UGF) in addition to the known nutrients above and the evidence has been summarised by Pike (1979).

In recent years fish meal has been regarded as too expensive for feeding to ruminants. However, the use of fish meal in highly productive ruminants (e.g. high yielding dairy cattle, lactating ewes and young rapidly growing calves) has been shown to be advantageous. In these cases, slowly degradable protein supplements are required, a high proportion of which will escape degradation in the rumen to be digested and absorbed from the small intestine. Of the protein concentrates, fish meal is among the least degradable (see Table 12) , yet has high digestibility in the small intestine, and have an amino acid pattern that is complementary to microbial protein and often similar to tissue needs.

The potential undegraded protein in fish meal is influenced by the method of handling, storing and processing of the raw material (Mehrez *et al.*, 1980). Some published degradability values for fish meal are listed in Table 12.

TABLE 12

Degradability in the rumen of protein from fish meal

Type of fish meal	Degradability %
Fish meal	29
Fish meal, Peruvian	0 - 31
Fish meal, White	61 - 63

* Miller (1978)

The energy value of fish meal is mainly derived from the protein and fat content, and is therefore of minor value as an energy source to the microbes during fermentation in the rumen. Fish meal is more valuable as a source of energy for the host animal, because its fat and protein content are mainly absorbed from the intestine.

Over the last few years there has been considerable interest in fish meal protein as a supplement for ruminant diets. It has been shown that, in young growing calves, supplementation of the basal diets with fish meal can lead to increases in live weight and intake. Whitelaw et al. (1964) investigated the relative value of different fish meal products (wite fish meal, herring meal, Peruvian fish meal and spray dried herring pulp). Their results indicated that live weight gain and nitrogen retention were significantly lower on the diet containing spray dried herring pulp than on either of the other diets. They suggested that protein solubility of fish meal product provides a reliable guide to their nutritive value for ruminants.

Since white fish meal was used in our trial, most of the work reviewed refers to that type of meal. Some conflicting results have been obtained by Bax and Offer (1983). They found that fish meal supplementation of a barley-based concentrate feed with grass silage had no effect on silage intake or ration organic matter digestibility although a significant increase in live weight gain was achieved over the unsupplemented diet. Otherwork has shown significantly increased ration dry matter digestibility (Bax and Offer, 1982) and a significantly increased silage dry matter intake (Garstang, 1981) with fish meal supplementation.

Ochoa and Owen (1983) used herring meal to supplement a basal diet of grass silage fed to Friesian calves of an initial live weight of 72 kg. Again supplementation increased growth rate and silage intake in a manner predicted by the ARC (1980). Chalmers et al. (1982) showed that young calves (7-8 weeks old) fed ad lib grass silage and 2.5 kg mineralised rolled barley responded to the addition of urea in one experiment but not in another; while in both experiments there was a consistent increase in live weight gain from supplementary fish meal over an initial 12 week experimental period. They suggested that the response obtained over this period could not entirely be due to extra energy intake or ration digestibility change but to an enhanced supply of UDP resulting from fish meal supplementation.

Comparisons of fish meal with urea supplementation tend to show that fish meal is superior to urea for protein supplementation, unless

the crude protein content of the diet is extremely low, i.e. below 85 g kg^{-1} DM (Smith et al., 1982). Miller (1973) showed that significantly more non-ammonia nitrogen reached the duodenum on a fish meal diet, than on a diet containing urea. Kirby and Chalmers (1982) reported an advantage in live weight gain by calves on the fish meal supplemented diet over those on a soya bean meal supplemented diet. However, supplementation with either soya bean meal or fish meal was not financially worth while. These results are in conflict with the results obtained by Righton (1981) which showed supplementation of silage with fish meal for calves of an initial live weight of 119 kg was economical.

Kay and King (1982) have also observed beneficial responses from fish meal supplementation; replacing 0.4 kg rolled barley by 0.4 kg white fish meal increased growth rate of British Friesian calves from 0.88 to 1.13 kg d^{-1}. However, in another experiment these workers fed 3 kg rolled barley containing either 15 g kg^{-1} urea or 60 g kg^{-1} white fish meal and did not obtain a significant increase in growth rate from protein supplementation. Kirby (1981) using formaldehyde-treated soya bean meal and two levels of fish meal supplementation all on an isonitrogenous basis, found significant differences in live weight gain with both levels of fish meal supplements being significantly greater than soya bean meal, but with no significant difference between the two levels of fish meal.

(b) Urea

Urea is a completely degraded non-protein nitrogen source - it has no UDP content and can only serve as a source of RDP. Because of its rapid degradation in the rumen the efficiency of microbial capture of urea nitrogen may be much less than 1.0. The ARC (1980) suggested a value of 0.8 for efficiency of capture which would reduce the RDP content of urea to 2300 g kg^{-1}. Urea provides no energy, vitamins, minerals or trace elements for the animal, and where it is used to replace conventional sources of protein care must be taken to ensure that satisfactory dietary levels of these nutrients are maintained by adequate supplementation. Also, care must be taken to avoid excessive urea intakes which could cause ammonia toxicity.

Although urea can form a useful protein source, there is evidence that where it forms a major part of dietary nitrogen, deficiencies of sulphur containing amino acids may occur. In such cases supplementation of the diet with a sulphur source may be necessary (Leibholz and Kang, 1973).

The main disadvantage of urea supplementation is the rapid release of NH_3-N from urea in the rumen after feeding which leads to two major problems. Firstly, the wastage of nitrogen, and secondly the ammonia toxicity. Another problem is the low palatability of the ration when urea levels exceeds 1%. There is no evidence for improvement of the palatability of urea - containing rations by inclusion of molasses and cobalt salts (Huber and Cook, 1972; Reid, 1953). Whenever possible, adaption to feeds containing urea should be practiced by the gradual addition of the supplement ration.

There have been several attempts to improve the value of urea by reducing its rate of hydrolysis in the rumen,but few have resulted in commercially usable products (see review by Bartley and Deyoe, 1977).

Experiments on the use of urea in calf diets have been made as long ago as the 1940s. Young ruminants apparently are able to make some use of urea even before their rumens have fully developed. Hart et al. (1939) fed young calves a ration containing 43% of its nitrogen from either urea or casein. Growth rate over 16 weeks was 0.59 kg d^{-1} for the calves fed urea and 0.68 kg d^{-1} for those fed casein. Higher levels of urea (66 and 70% of the total -N) caused diuresis.

Two month old calves did not grow when fed a diet containing only 4.4% protein. When urea was added to the diet to give a calculated protein content of 16.2%, calves increased in body weight at fairly satisfactory rates, but not equal to those fed a normal ration (27.7 kg v.s. 36.4 kg for 2 months). The added urea increased digestibility of dry matter and carbohydrate and enabled the calves to remain in positive nitrogen balance (Lossli and McCay, 1943).

Brown et al. (1956) conducted a similar experiment by comparing a starter containing a conventional protein supplement (linseed meal) with both a low protein starter and a urea-containing starter. All groups of calves grew at comparable rates over the first six weeks, while the whole milk was being fed. When whole milk feeding was discontinued, the urea-supplement and conventional protein groups grew significantly faster than the low protein group. No significant differences in either gain or starter consumption were detected between the urea and the conventional protein groups. Similarly calves weaned at 7 weeks of age and given a urea supplement to a

basal mixture containing 6.7% crude protein utilised appreciable quantities of urea to meet their requirements for growth as early as the sixth week of life (Brown et al., 1960). Leibholz and Kang (1973) found that supplementing urea-containing diets with sulphur resulted in significant increases in live weight gain and intake. They suggested that sulphur supplementation improved micrbial production of methionine, thereby improving the amino acid profile of the protein reaching the small intestine.

The above workers (Leibholz and Kang, 1973) showed that intake of diets containing urea was less than that of isonitrogenous diets containing true protein supplements. This low intake was attributed to the reduction in palatability associated with urea supplementation. Huber and Cook (1972) showed that mixing the urea with molasses improved palatability as well as energy supply, although no palatability problem was reported by Leibholz and Nayler (1971). They showed that growth of calves was not reduced when up to 40% of the dietary nitrogen was in the form of urea.

Chalmers et al. (1982) reported small response in live weight gain due to urea supplementation over the basal diet of ad lib grass silage and mineralised barley concentrate, during the first twelve weeks of the trial using 7-8 week old calves although fish meal gave a bigger response. Kay et al. (1967) using Friesian calves of an inital live weight of 50 kg, found significantly greater live weight gain, and feed conversion efficiency for the animals on the fish meal diet over those on the urea diet. As fish meal was replaced by urea in the diet, nitrogen retention fell, rumen ammonia concentration and blood urea increased. Nitrogen retention was lowest when fish meal was completely replaced by urea. These results demonstrate that urea is rapidly degraded in the rumen so that the micro-organisms cannot capture all the ammonia produced and incorporate it into microbial protein. This means that the ammonia is lost with respect to the animal.

Stobo et al. (1967c) also reported that growth of young calves was reduced by 20 to 30% when urea supplied 40% of the nitrogen in the ration Smith et al. (1982) concluded that responses to urea supplementation will increase as dietary crude protein concentration falls below 85 g kg^{-1} dry matter.

Conclusions

The animal responses to supplementation with urea or fish meal which has been described support the following conclusions:

1. Urea supplementation is only beneficial if the rumen microbial population is N (NH_3) deficient. Urea acts as an RDP source stimulating microbial protein synthesis, digestion and intake.

2. In rapidly growing calves of less than 200 kg live weight fish meal almost always proves superior to urea as a supplement. For such animals it is not enough just to meet the microbial needs for RDP and thus the high UDP value of fish meal is decisive and may often be financially worth while.

3. In cattle of more than 200 kg live weight, the difference in response between urea and fish meal is inconsistent. Urea gives response only when the basal diet is RDP deficient, but fish meal may improve live weight gain even when this is not the case. Fish meal responses of this type are not totally reproducible, but are usually associated with increased ration intake and digestibility. The exact mechanism of these responses is unknown.

SECTION 4

FISH SILAGE

Introduction

At no time in human history have food shortages been as widespread and affected as many people as they do today. As estimated five hundred million people in the world are malnourished (NAS, 1977) and the food supply problem will become more severe as the world population rapidly grows from the present level of nearly five thousand million to 12 to 16 thousand million by 2150 (UN, 1973).

The basic problem then, is how to provide such a food supply in the face of increasing population and diminishing resources needed to produce this food. Therefore, in planning for the coming decades we need to consider not only the present conditions affecting food production, but the many constraints that may impede our achieving these goals in the future. An important factor is the competition between the human and animal population for foods such as barley, corn, soya bean, etc. There is an essential need to look for new foods that could become available for animal diets, so as to spare such foodstuffs for direct human consumption. By-products of several industries may be considered as a possible basis for animal diets, especially for ruminants (cattle, sheep) which have the ability to utilise fibrous foods and do not require large quantities of high quality protein.

These include wastepaper and other wood products provided they are free from toxic contamination. Another possible source of high-fibre material is livestock solid waste. Poultry droppings have already been used, primarily as a protein source and recent indications are that pig dung can be used as a forage replacement. One of the drawbacks of the use of such material is that, if it is to be widely available, it needs to be dried, and this of course markedly increases the cost of the product.

The recent emphasis on the role of undegradable protein in ruminants animal feeding may well give great impetus to the incorporation of processed animal by products into cattle diets, for example fish

meal, meat meal. Biotechnology is a field that is being studied and which may eventually yield products of great value to the animal feeding industry. Selected microbes are grown on suitable media (based on oil product) to give a high quality material suitable for use in the diets of calves and lactating cows. This development has been severely limited by the increases in world oil prices.

Fishing operations around the world involve considerable wastage of fish offals and by-catch fish available for use in animal feeding. High transport costs or material losses may be incurred in sending this material to the fish meal factories, and it is frequently economically unfeasible unless large quantities of high quality waste are available or the distances are small. An alternative scheme is to make fish silage for animal feeding.

It has been known for many years that waste fish can be preserved as a kind of silage, and much interest has been shown in processes which enable utilisation of this waste in animal foods.

What is fish silage

There is no one term which adequately describes preserved fish waste but throughout this work the term 'fish silage' is used rather than liquid fish protein (LFP).

Fish silage is defined by Tatterson (1976) as a liquid product made from whole fish or parts of fish that are liquefied in the presence of added acid, by the action of enzymes naturally present in the fish. The enzymes breakdown fish proteins into smaller soluble units, and the acid helps to speed up their activity while preventing bacterial spoilage during processing and storage. Fish silage can be made from most fish or marine species.

The potential for using fish silage in the diet of farm animals and many balance trials, have indicated that fish silage has a high nutritive value for poultry, growing pigs, growing calves and for fur animals.

Use of fish silage past and present

The production of fish silage from by-catch fish or fish-waste was

developed originally in the 1920s in Finalnd by A.I. Virtanen, who treated green fodder with a mixture of sulphuric and hydrochloric acid which he called AIV-acid. Work on fish silage started in Sweden in about 1936 when Edin conducted an experiment using AIV-acid, sulphuric acid, molasses and formic acid.

In 1940 Edin carried out further work using AIV-acid and suggested that the amount of AIV-acid required for the preservation of different types of fish and fish-waste could be calculated according to a formula based on the protein and ash content of the material. The formula was intended to yield a mixture with a pH value of 2.0, which had been found necessary to ensure satisfactory preservation. If the correct pH was not achieved a further quantity of acid had to be added according to a second formula which took account of the amount of acid already added and the resultant pH value of the mixture following the addition of the first quantity of acid. Based on this formula, Olsson (1942) set up a table giving the amount of acid required for various Scandinavian fish raw materials.

With the outbreak of the second world war hydrochloric acid became very scarce and Olsson (1942) looking for other alternative preservatives, tried formic acid. The reason for his choosing this acid is not known, but at a relatively high pH it limits the growth of bacteria to a greater extent than other organic acids. Acetic acid was at that time about the same price as formic acid, but its preservative action is somwhat less, whilst propionic acid, although having a good preservative action, was likely to be considerably more expensive.

Olsson's early work with formic acid was carried out on Baltic herring. He studied the variation of silage pH with acid concentration and examined the keeping qualities of the different mixtures (Olsson, 1942).

The chief advantage of AIV-acid over the other method is its cheapness, but this is probably out-weighed by the disadvantage in that it is a highly corrosive liquid producing a corrosive product which requires neutralisation before feeding to the animals. The production of fish silage on an industrial scale started in Denmark in 1948 and, although techniques have been modified a little, a

thriving industry still exists there. Subsequently, substantial amounts have been produced in Poland (Sikorski, et al. 1969), Denmark (Jensen, 1973) and other European countries for use in pig and poultry feeding. More recent developments, which have been reported by Tatterson and Windsor (1974) in the UK have indicated a renewed interest in fish silage in recent years.

The production of fish silage using formic acid involves grinding the fish or the fish-waste and adding 3% (w/w) formic acid in such a concentration that the final product will have a pH of around 4 (Tatterson and Windsor, 1974). But such silage sometimes became infected by moulds, in particular Aspergillus flavus (Gaiger, 1978; Kompiang et al. 1980). Gildberg and Raa (1977) developed a promising procedure for the processing of fish silage. The procedure may be divided into three stages as briefly described below.

1. Preservation - Fish-waste is minced and preserved with 0.75% formic acid and 0.75% propionic acid (v/w).

2. Autolysis - Acid-preserved fish-waste is kept at 30-33°C for three days; proteolytic enzymes in the fish-waste cause autolysis and liquefaction of the material.

3. Removal of fat - Following heating to 95°C for five minutes a major part of the fat in the autolysate is removed by centrifugation. This method has subsequently been adapted for use on an industrial scale by many Scandinavian countries.

Subsequently, Kompiang et al. (1979) showed that 1.5% (v/w) of the 50:50 mixture of formic acid and propionic acid as prescribed by Gilidberg and Raa (1977) was not sufficient to produce stable silage of minced by-catch fish. However, stable silages with a fresh acidic smell were always obtained when 3% (v/w) of propionic acid/formic acid (1:1) was added.

The advantage of using the propionic acid/formic acid mixture was evident when the silage was mixed with a carbohydrate carrier.

It has been demonstrated that waste-fish can be preserved by lactic acid bacterial fermentation as a silage (Raa, 1965; Kompiang et al.

1980; James et al., 1977; Raa and Gildberg, 1982).
This method has some advantages over chemical silage at least for countries where the acids are still imported, rather hard to obtain and the price is high.

The renewed interest in fish silage is related to the desire to make the maximum use of trash fish and fish offal in situations where the quantity involved or the transport costs prohibit conversion into fish meal. This situation also occurs in small fishing communities remote from the main fish processing centres in both Europe and tropical countries. Feeding trials have shown that the inclusion of fish silage in barley based diets for pigs will give satisfactory live weight gain and food conversion efficiencies (Hillyer et al., 1976).

The same results have been obtained when fish silage has been used as a source of protein for supplementing hay, straw and grain to provide a balanced winter ration for cattle (Young and Dunn, 1975).

Source of raw material for processing fish silage manufacture

A. Processing operations

The production of fish fillet on a commercial scale involves a considerable loss of waste materials. Common fillet yields vary depending on fish species, size, type and usage of processing equipment, but a 35% yield is probably a representative average (Bucove and Pigot, 1976).

Some additional flesh (10-15%) from backbones, fins and lungs may be recovered with deboning machines (Mackie, 1974). The remaining by-product or waste are filleting scrap consisting mainly of the clavicula, heads, backbone, ribs and tail, with adherent flesh with or without viscera. On factory freezing trawlers the viscera are used together with the filleting scrap to produce fish meal, but for the most part viscera are discarded (e.g. stomach, intestine, spleen, liver, milt, gonads together with discarded roe). According to Reay et al. (1943) the proportion of viscera in Cod (*Gadus morrhua*) and Haddock (*Gadus merlangus*) is in the order of 6-12%, of which the liver may represent about half. At spawning, the gonads

may account for 15% of the weight of the whole fish, thus increasing the amount of viscera to as much as 22%.

Wignall and Tatterson (1976) stated that the guts amounted to about 6% of the total weight of the fish.

Various methods have been proposed for the handling or processing of fish viscera.

(a) Frozen storage of unprocessed viscera and subsequent use as a protein source in mink and fox diets have been employed to a certain extent. However, freezing is an expensive method and is further limited by lack of freezing capacity. In addition, there have been arguments against incorporation of viscera and even whole fish in fur animal diets (Ahman, 1969). The main concerns have been the high content of bacteria and enzymes in the gastro-intestinal tract. As a consequence, fish viscera may be of a poor hygienic quality, low storage stability because of the high content of unsaturated fatty acid may cause rancidity problems upon prolonged frozen storage.

(b) Where facilities are available, reduction of the viscera to fish meal is a useful processing method. But the production involves technological difficulties in the handling and drying of the material. Viscera are difficult to process because they autolyse readily, are difficult to handle in a screw press after cooking. Although pressing is necessary with demersal species (members of the shark family) for oil extracting. Because of these technical problems little effort is made to process fish viscera into fishmeal (Olley _et al_., 1968).

Fish waste from processing industries such as tuna canning can also be a very good source of raw materials for the production of fish silage. Offals and trimmings for such plants could become an important source of raw material for silage.

B. Industrial fish

Industrial fish can be defined as unconventional or unfamiliar species may be caught which cannot be used in processing or sold on local markets. Therefore most of it is converted into animal feed.

In 1977 up to 180,000 t of such fish (sand eels, summer sprat and scad, together with whatever winter sprat and mackerel were not required for human consumption) were converted into fish meal in the UK. During the same year world landings of industrial fish were approximately 20,000,000 t (Tatterson et al., 1979).

Shrimp trawling operations, principally in South East Asia and Latin America, leads to great wastage. Substantial quantities of by-catch fish are thrown overboard during shrimp trawling because the trawlers are too small to carry the quantity involved or because they are not marketable. (Meinke (1973); IDRC (1974) suggest that the by-catch fish in Thailand amounts to 400,000 t per annum and 200,000 t in India. These countries can ill afford to waste such enormous quantities of fish. Ironically countries in this situation are often importing substantial quantities of fish meal to support their expanding animal production industries. Also in the small-scale fisheries in the tropics, daily or seasonal gluts of fish are common. Because of transport difficulties and inadequate processing facilities this surplus fish is often under-utilised.

What is often required is a flexible, low cost system capable of handling regular but variable quantities of fish waste and by-catch fish at relatively low levels of throughput. The flexibility of such a process is vital when one considers the nature of fishing operations, since the quantities of raw material available for processing varies widely from day to day. The fish silage process can be applied in this kind of situation.

Production and Utilisation of Fish Silage

Fish is one of the most important sources of protein in the world. Many preservation methods have been developed, such as drying, salting, fermentation, freezing and canning. In spite of these practices, considerable wastage occurs through spoilage resulting from inadequate storage, handling and processing systems and the discarding of by-catch fish in the sea. Research has therefore been initiated to develop alternative methods for preserving and utilising fish wastes.

Ideally the method used should be inexpensive and easily adapted for

use at any level of production. Production of fish silage appears to fulfil these requirements.

The principle of production is the preservation of material by preventing microbial degradation by using one of two methods.

(a) By adding acid, inorganic and/or organic, which lowers the pH sufficiently to prevent microbial spoilage. The fish silage became liquid because the tissue structures are degraded by enzymes naturally present in the fish.

(b) By microbial fermentation, by mixing the fish with a fermentable sugar which favours growth of lactic acid bacteria.

I - Acid Preserved Fish Silage

(a) Inorganic acid

The acid ensilage of fish waste was developed originally from a method invented by A.I. Virtanen in the 1920s. Sulphuric and hydrochloric acids (AIV) were used to acidify fish waste and the product was neutralised with chalk before feeding. Because of the high buffering capacity of fish, due to high levels of protein and minerals, more acid is needed to produce a stable fish silage than a green forage silage.

Edin (1942) found that the amount of AIV acid required for the preservation of different types of fish and fish waste could be calculated according to a formula based on the protein and ash content of the material.

$$(a \times 0.14 + b \times 0.9) \text{l of 14 N acid per 100 kg raw material}$$

where a = % crude protein of wet weight,

and b = % ash of wet weight.

The formula was intended to yield a mixture with a pH value of 2.0, which had been found necessary to ensure satisfactory preservation.

Before mineral acid silage can be fed to animals it must be neutralised. Acording to Petersen (1953) it is advisable to add 2 to 5 kg chalk per 100 kg of silage.

In work carried out by Freeman and Hoogland (1956) in Canada, using cod and haddock offal, sulphuric acid was used successfully.

Tülsner (1960) described laboratory work carried out in Germany using sulphuric acid and commented on the factors influencing the efficiency of the process. In 1970 a German patent described a method of using hydrochloric acid and heat, which it is claimed has the advantage of speeding up digestion considerably. However, the high salt level which results from neutralisation is nutritionally undesirable and the neutralisation procedure is so laborious as to be a major practical problem.

The chief advantage of AIV acid over the organic acids is its cheapness, but this is probably outweighed by the disadvantages described and in addition by the fact that it is a highly corrosive liquid.

(b) Organic acid

With the outbreak of the second world war hydrochloric acid became very scarce and Olsson (1942) looking for other preservatives, tried formic acid. This acid limits the growth of bacteria at a relatively high pH.

Olsson (1942) also demonstrated that the ash content of the raw material was an important factor in deciding how much acid would be required for adequate preservation. He treated fish mixtures of varying ash content with different amounts of acid and by noting whether or not preservation was affected, produced figures relating ash content to amount of preservative required. His results are shown in Table 13 and an approximate equivalent in percentage by weight of acid is included.

TABLE 13

Formic acid required to preserve fish of different ash content

Ash content of material %	90% Formic acid required	
	L per 100 kg fish	% by weight
2.0	0.85	1.02
2.5	1.00	1.20
3.0	1.15	1.38
3.5	1.30	1.56
4.0	1.45	1.74
4.5	1.60	1.92
5.0	1.75	2.10
5.5	1.90	2.28
6.0	2.05	2.46

*Olsson (1942)

Based on these results he derived a formula:

$$L = 0.25 + 0.3 \times \% \text{ Ash}$$

where L = The amount of organic acid required. (Litre)

N.B. Olsson's work with formic acid was carried out on Baltic herring.

Hanson and Lovern (1951) suggested the use of formic acid alone as being less corrosive than stronger mineral acids, and having a very good bacteria static action. Petersen (1953) and Lovern (1965) have reviewed a number of different combinations of acids and their relative merits.

The preservative acid chosen in recent work in the UK was an 85% solution of formic acid. To completely prevent the growth of spoilage bacterial moulds and pathogenic bacteria (such as Salmonella and Clostridum-botulinum) a pH value of 4.0 was chosen (Tatterson, 1976). This pH was achieved by the addition of 3.5% by weight of 85% acid to material containing appreciable amounts of bone, such as white fish offal. With whole fish, somewhat lower pH values resulted. However, such silage sometimes became infected by moulds, in particular Aspergillus flavus. This has been reported by Gaiger (1978) and Strøm et al. (1980). Norwegian researchers have lately developed a method for production of a low-fat fish silage (Raa and Gildberg, 1976; Gildberg and Raa, 1977). The procedure involves preservation with 0.75% formic acid (v/v) and 0.75% propionic acid (v/v), heating to 30-33°C for three days to promote analysis by indigenous proteolytic enzymes, followed by heating to 95°C for five minutes with subsequent removal of fat by centrifugation. The process has subsequently been adapted for industrial scale production in Norway (Johnsen and Skrede, 1981). The advantage of using the propionic acid/formic acid mixture was evident when the silage was mixed with carbohydrate carrier. Moist mixtures of one unit weight of silage and one unit of dry cassava, or corn meal, remained free of mould growth for at least three months at 30°C. A corresponding moist mixture of the formic acid silage spoiled, usually in a few weeks (Kompiang, Arifudin, and Raa, 1980). They obtained stable silages with fresh acidic smell when 3% (v/w) of propionic/formic acid (1:1) was added. The other advantage of the new procedure was the removal of lipid which imposed its nutritional value markedly. Although organic acids are more expensive than mineral acids, the difference in price is partly counterbalanced by the higher efficacy of the organic acids, and it is not necessary to neutralize a silage produced with organic acids.

The anti-microbial activity of weak organic acids is associated

with the undissociated molecule. Hydrogen ions (protons) cannot diffuse through cell membrances. Living cells can therefore maintain a neutral pH in the cytoplasm, even in an acid environment. Protons may nevertheless be carried into the cell byweak organic acids, provided that the pH in the environment is acid. At low pH an organic acid willexist in its non-charged undissociated form, which can freely pass cell membranes. Inside the cell, where the pH is neutral, the weak acid will immediately dissociate and both the anion and the proton become trapped in the cytoplasm. The internal pH of the microbial cell therefore gradually falls, and the anion accumulates. Both these factors contribute to the antimicrobial effect of weak organic acids. The anion may, however, be metabolised by the cell, which in this way counteracts its toxicity. This probably happens with acetate, which has a considerably lower antimicrobial activity than propionate and formate (Raa et al., 1983)

This theory of the mode of action of weak organic acids, implies that their antimicrobial activity should increase with falling pH, according to their acid/base titration curve. This has in fact been demonstrated using the carcinogenic fungus Aspergillus flavus as the test organism by Strøm et al. (1979).

Propionic acid, which has a pKa of 4.9 inhibited this fungus at pH values below 5. Whereas formic acid having a pKa of 3.7, inhibited below pH 4. In a medium lacking organic acids, the same fungus grew fast even at pH 2. Consistent with the theory is the fact that a stable silage can be obtained at a higher pH with propionic (Gildberg and Raa, 1977) than with formic acid (Tatterson, 1976). Also the work of Hansen (1959) on the preservative efficacy of organic acids supports the theory above. The pH of moist mixtures of fish silage and grass meal was adjusted to 3.5 to 4.0 with sulphuric acid, and the quantity of organic acid needed for satisfactory preservation was recorded as follows:

acetic acid, 2.0%; formic acid, 1.5%; propionic acid, 0.4%; benzoic acid, 0.4%; ascorbic 0.1%.

These data recommended the use of propionic acid in order to prevent growth of hazardous fungi.

(c) Mixturesof inorganic and organic acids

Organic acids are more expensive than inorganic acids. To reduce the price of preservation of fish with high buffering capacity, a mixture of inorganic and organic acid can be used; the cheap inorganic acid will lower the pH so that the organic acid becomes antimicrobial. Such experiments have been carried out mainly with formic acid in a mixture with sulphuric. Lisac (1961) considered in detail the use of various acid mixtures in varying quantities on the liquefaction and keeping quality of silage produced from Mediterranean sardine offal.

Lovern (1965) has investigated the most cost-effective mixtures of mineral and organic acids.

The production of fish silage using formic/sulphuric or formic hydrochloric has increased in Denmark (Jensen, 1973) and in Poland (Sikorski, 1969). Approximately 3% (v/w) of a 3:1 (v/v) mixture of sulphuric and formic acids preserved fish offal equally well as 4% (v/w) of pure sulphuric acid (Olley, 1982). Sulphuric acid can be replaced by hydrochloric acid (Disney and Hoffman, 1976) or phosphoric acid (Jensen and Schmidtsdorff, 1977).

Table 14 summarizes some experiments carried out with formic or sulphuric acid or mixtures of the two acids.

Because of the higher ash content of tropical by-catch fish, more organic acid is needed for preservation; 2.5% formic acid or a mixture of formic and propionic acid being the minimum concentration which ensures preservation (Kompiang, Arifudin and Raa, 1980). Therefore, it is important for the viability of the silage process to find the most economic combination of acids for such raw materials.

TABLE 14

Storage stability of fish silage preserved with formic acid, sulphuric acid and a mixture of these acids

Fish material	Acid added (% v/w of pure)		pH		Keeping time days
	H_2SO_4	HCOOH	Initial	Final	
Baltic herring offal	-	0.9	5.0	-	60
*Ollson (1942)	-	1.35	4.4	-	> 160
	-	2.25	4.0	-	> 160
White fish offal	-	2.1	4.1	4.6	> 120
	3.0	-	2.8	4.4	30
*Atkinson _et al_ (1974)	4.0	-	2.2	4.7	> 120
	1.5	0.85	4.3	4.5	60
	2.5	0.85	1.2	3.9	> 120
Sardine offal	1.8	0.9	-	-	> 180
*Lisac (1961)	2.15	0.7	-	-	> 180

Physical processing

(a) Choice of acid

In the preparation of an acid silage the choice of preservative is between mineral or organic acids used either alone or in combination.
The choice will depend upon the cost and availability of the acid and the conditions under which the product is prepared. For reasons of safety, reduction of the amount of acid was felt to be inadvisable. Also, for simplicity, different amounts of acid for various materials were thought to be undesirable.

(b) Chopping or mincing

The material should be reduced in size preferably to pieces no larger than 3 to 10 mm in diameter. Mincing or chopping is necessary to distribute the enzymes throughout the mass of material and also to assist blending of the acid to avoid pockets of untreated fish where bacterial growth can continue. Equipment used for silage chopping can vary considerably. On the small scale it might be sufficient to pulp the raw material. For larger scale production, however, a mincer capable of reducing material to the required size is essential.

(c) Blending with acid

It is common practice to mince the fish and mix it with the preservative acid. The mixing of mince with the acid may be a significant practical problem if the fish is fresh, because the muscle components become rubber-like when exposed to acid. The mince, therefore, tends to form closed pockets where the acid does not enter quickly enough to prevent spoilage. Though if the raw material is not fresh, this is not a major problem. However, the solution is not to let the fish spoil before the acids are added, but rather to cut it or chop into pieces before the acid is added. This will ensure good preservation because the acid will sterilise the fish surface and the guts which carry the spoilage microbes. In practice it may be advisable, however, to mix the newly chopped or minced fish with the liquid silage from the storage tank to make the mixture pumpable. This serves also to obtain an efficient mixing of fish and acid.

Such problems are not encountered with fish tissues with low muscle content, such as guts, and with small fish which are mixed with the preservative acids (Kompiang, Arifudin and Raa, 1980).

The addition of acid to whole or chopped small fish may even facilitate efficient extrusion of oil because skin dissolves while muscle contracts in acid (Gildberg and Raa, 1979).

The addition of the acid lowers the pH sufficiently to prevent microbial spoilage and to create suitable conditions for the autolytic enzymes always present in fish. These convert fish protein into smaller soluble units such as peptides and amino acids. The equipment required for mixing the acid with the raw material depends upon the scale of production. It must be acid resistant and sufficiently powerful to ensure that a homogenous mixture of fish and acid is made. For safety, a pump or measuring device for handling acid is advisable.

Liquefaction

Little is known about the mechanism of autolysis of fish tissue in a silage. The liquefaction occurring during the process of ensilage is certainly the result of enzymatic action, since cooked fish does not liquefy.

A silage gradually liquefies due to the activity of tissue degrading enxymes which are naturally present in the fish mainly in the digestive organs (Tatterson and Windsor, 1974; Backhoff, 1976; Gildberg and Raa, 1979; Raa and Gildberg, 1976; Hjelmeland and Raa, 1982).

The enzymes are spread throughout the fish mass after grinding (Meinke and Matil, 1973). This is supported by the fact that a silage of fillet alone liquefies poorly (Tatterson and Windsor, 1974)

The factors which affect the rate of liquefaction are:

1. the activity of digestive enzymes in the raw material;
2. the physiological condition of the fish at the time it was caught;
3. the pH;

4. the temperature;

5. the preservative acids.

Since the protein is the major structural component in the fish tissue, it is mainly the proteases which are responsible for autolysis. The digestive proteases have an optimum in the pH range 2 to 4, when assayed with haemoglobin as substrate (Raa and Gildberg, 1976).

Although the precise pH response may be different when fish tissue rather than haemoglobin is used as substrate (Gildberg and Raa, 1979)

Raa and Gildberg, 1976 found that the rate of autolysis was highest at pH 3.5-4.0. However, the pH optimum for autolysis may of course vary with the substrate used.

The rate of autolysis is affected primarily by the pH and not by the type of acid used for preservation. This was demonstrated by measuring the rate of tissue degradation in silages with different pH but constant concentration of propionic acid. The rate of autolysis and the yield of soluble matter was markedly lower at pH 3 than at 4 (Raa and Gildberg, 1976) see Figure 6. This may explain why a herring silage autolysed better if formic acid was used alone rather than mixed with sulphuric or phosphoric acid because pH was 4.5 in the former case, but 3.1 in the latter (Jensenn and Schmidtsdorff, 1977). Therefore, organic acids have to be used to obtain optimum solubilisation.

Most active in acid conditions are pepsin-type stomach proteases, but certain lysosomal enzymes like cathepsin D, which is a major muscle protease, may also be of importance. Collagen degrading enzymes are probably present in fish tissue(Yoskinaka, Sato and Ikeda, 1978; Dugal and Raa, 1978). However, the importance of these enzymes in autolysis is doubtful. The denaturation temperature of collagen decreases with increasing acidity (Hayashi and Nagai, 1973), and fish collagen is certainly denatured and rendered susceptible to degradation by ordinary proteases under normal ensiling conditions.

It is noteworthy that insoluble sediment always remains in a fish

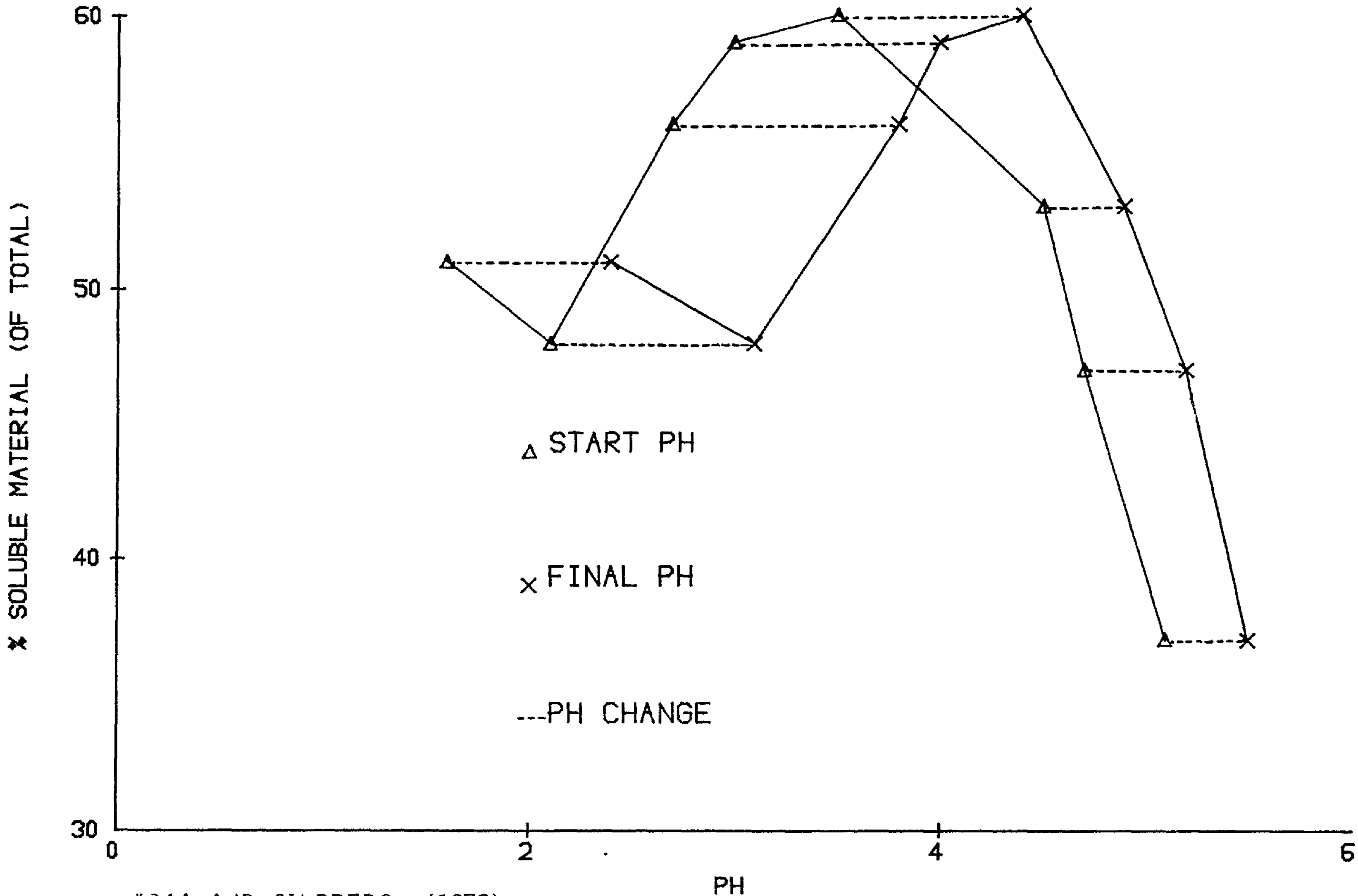


FIGURE 6: AUTOLYSIS OF COD VISCERA AT DIFFERENT PH

silage so autolysis is never total (Tatterson and Windsor; Freeman and Hoogland, 1956). Also when commercial enzymes were added in order to solublise fish tissues, an insoluble residue remains (Hale, 1969; Freeman and Hoogland, 1956). It is not known why a certain proportion of the fish tissues resists enzymatic digestion. It may be that the rate of reaction becomes very slow when the most favourable binding sites on the substrate have been used. These are indications that pepsin-type enzymes are most active on substrate proteins which contain high amounts of hydrophobic and basic amino acids (Olley and Pirie, 1968). Experimental results show, however, that the residual fraction of fish silage has a normal or even high content of such amino acids (Gildberg and Raa, 1979). It seems that certain protein fractions are resistant to proteolytic degradation at constant pH. Protein digestion of fish tissue by living fish is however very efficient (digestibility more than 95%) but this does involve a two-stage process at both acid and neutral pH.

It is not yet clear whether fish muscle, which has been shown to contain trypsin inhibitors (Hjelmeland and Raa, 1980) contains in addition protease inhibitors which retard autolysis at acid pH. Available data does not indicate such inhibitors exist.

Digestive proteases from fish have optimum acitvity at 45 to 50°C with haemoglobin as a substrate. The optimum for autolysis is slightly lower (Gildberg, unpublished). Temperature above 50°C will cause inactivation of the enzymes. It is however difficult to estimate to what extent the optimum is determined by thermal protein denaturation or self-digestion of the enzymes. Strangely, there are little differences in the temperature optima of proteases from arctic fish, which live their entire life below 4°C and tropical fish which live at 25 to 30°C.

Raa and Gildberg (1976) studied the autolysis of ensilaged cod viscera at different temperatures. They found that the rate of autolysis was strongly enhanced at temperatures above room temperature. The rate of autolysis increases very markedly within a rather narrow temperature range. This may be because thecollagen in the connective tissue becomes heat denatured and thus more susceptible to proteolytic degradation. The protease activity in the silage

is unchanged after several months at 27°C.

Freeman and Hoogland (1956) studied the autolysis of fresh cod and haddock viscera in the presence of sodium nitrite as a preservative. They found that at 37°C autolysis progressed rapidly; at 25°C slightly more slowly, and at 15°C it had become very slow. They suggested that in practice autolysis at 37°C or at 25°C could be used, the selection of a temperature would probably depend on the processing cost and the availability of heating equipment. Silage made from fresh white fish offal takes about two days to liquefy at 20°C, but takes 5-10 days at 10°C, and much longer at lower temperatures (Tatterson and Windsor, 1974). Thus in winter it would be necessary to heat the mixture initially, or to keep it in a warm area until liquid. If the temperature of the silage is not initially raised by the application of direct heat, liquefaction will occur only after a period of weeks or even months.

The rate of liquefaction depends also on the type of raw material and particularly its freshness. Most species can be used, but sharks and rays are rather difficult to liquefy, and should be mixed with other species. Fatty fish liquefy more quickly than white fish offal, and fresh fish liquefy much more quickly than stale fish (Tatterson and Windsor,1974).

About 80% of the protein in an acid fish silage usually become solubilised after one week at temperatures around 23 to 30°C (Gildberg and Raa, 1977; Tatterson and Windsor, 1974). The yield of solubilised protein may vary significantly, depending on the raw material; flesh gives the lowest yield, viscera the highest (Backhoff, 1976; Tatterson and Windsor, 1974). The protein solubilisation may also be reduced by a high level of lipid in the fish which inactivates the proteolytic enzymes (Raa and Gildberg, 1976). This might explain the resistance of the sediment which contains a high level of lipid to further enzyme digestion.

Removal of oil

When low oil content raw material, such as white fish offal, is used to produce fish silage there are no problems in utilising the finished product to replace fish meal in the diet of pigs. There is no risk of taint in the carcase provided the fish oil content of the whole ration is not greater than 1% on a dry matter basis (Adamson and Smith, 1976). At a 10% dry matter inclusion rate this corresponds to a maximum oil level in the fish silage of approximately 2% in the fresh matter. However, the amount of low oil content material such as white fish offal available in the UK has decreased dramatically over recent years and almost all of it is used for the manufacture of fish meal. Furthermore, the quantities of low oil content material are likely to become even smaller in the future.

The oil content of fish vary depending on species and season, and many of the silages made from raw materials containing a high level of oil such as herring, sand eels, sprats and pelagic fish, will be of little value for feeding unless the oil content is reduced. The use of high oil content silage is limited by the high content of unsaturated fatty acids which may cuase digestive upset and rancidity and palatability problems upon prolonged storage.

A method of obtaining low-fat fish silage has been described (Raa and Gildberg, 1976; Gildberg and Raa, 1977; Johnsen and Skrede, 1981). The procedure involves preservation with 0.75% formic acid (v/v) and 0.75% propionic acid (v/v), heating to 30-33°C for three days and autolysis by indigenous proteolytic enzymes, followed by heating to 95°C for 5 min with subsequent removal of fat by centrifugation. This method has subsequently been adapted for industrial scale production by the Fishery Technology Research Institute in Tromsø, Norway. For silages prepared from oily material, the general technique is the same as that outlined for white fish, but an additional oil separation stage is required. Oil removal should be carried out as soon as the silage is liquid enough to be pumped, usually within 1-2 days. The temperature is first raised to the required temperature (65 to 95°C) which can be achieved by the use of a simple heat exchanger or by supplying direct heat. When the required temperature is reached, coarse, suspended solids are removed by decanting followed by centrifugation for oil separation.

Although coarse solids might be removed by screening. These operations yield:

1. decanted solids;
2. de-oiled silage;
3. oil;
4. centrifuge desludge.

The relative proportion of each fraction depends on the composition of the raw silage and the state of digestion. Figure 7 illustrates an approximate mass balance for silages made from herring offal, originally containing about 16% oil.

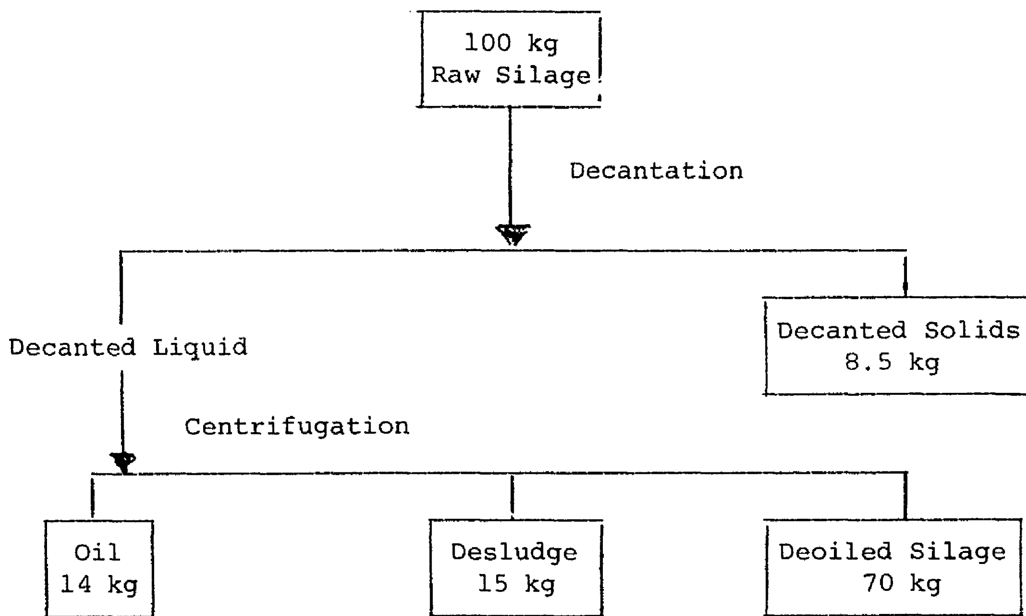


Figure 7: Silage fractions after de-oiling

*Tatterson (1976)

It is seen that the total weight of the separated fraction is 107.5 kg and theextra weight is accounted for by the presence of water added to the solids in the centrifuge bowl (desludge) when automatic cleaning occurs.

If decanted solids and deoiled silage are mixed to form the final product, the figures show that 1 tonne of herring offal containing about 16% oil, yields 785 kg silage and 140 kg oil. Oil recovery

is a little under 90%.

The deoiled silage contains little oil (approximately 1.5% and about 15% protein and can be fed at realistic levels (Tatterson, 1976).

Investment in deoiling equipment may be viable also from an economic point of view, despite high capital cost. One prerequisite for successful operation of the deoiling equipment is that the degree of autolysis is high - otherwise a high proportion of the oil will be retained in the sludge phase, and the yield of the deoiled solubles will be low. A high volume of sludge will, moreover, create technological problems due to emulsification. With ensiled fish viscera, which autolyses more than 90%, this was not a problem, and the system, described by Størm et al. (1980), worked satisfactorily to produce silagewith an oil content of 0.4 to 0.3% of wet weight. Further information about large-scale plants for deoiling can be obtained (Anon, 1979; Poulter et al., 1980). In small-scale situations it may be satisfactory to rely on self-sedimentation and manual decanting of the oil (see Figure 8).

Storage

Fish silage of the correct acidity keeps at room temperature for a least one year without putrefaction, Gildberg and Raa (1977). In practice it would probably not be stored commercially for more than about six months (Tatterson and Windsor, 1974). It is useful to have a knowledge of the chemical changes taking place during storage.

1. Changes in protein

During the storage of fish the protein is broken down by enzymes and the nitrogen in the silage becomes more soluble. Results for different silages vary little, and within about a week, provided storage temperatures are high enough, up to 70% of the nitrogen present will be soluble. For both white fish offal and that made from oily fish, the solubility of nitrogen has been found to be temperature dependent (Disney et al., 1977).

Figure 9 shows results obtained from silage prepared from sprats after 20 days storage. The rapid increases in protein breakdown during the first few days storage for material held at the higher temperatures can be seen.

Tatterson and Windsor (1974) also investigated the changes occurring in the protein during long periods of storage, using a wide range of raw material (sprats, herring, herring offal, sand-eels, white fish offal and mackerel) mixed with 3% by weight of 98% formic acid. Silages were stored for a period of one year at temperatures of +2°C and +23°C. These two temperatures were chosen as representing the probable extremes of temperature at which fish silage might be stored commercially in the UK. They found that with both white fish and sprats the percentage of soluble nitrogen in the freshly prepared silage amounts to between about 10 and 20% during the first few days. Subsequently the levels in the samples stored at 23°C rises very sharply to about 75% after 10 days and 80-85% after 30 days (see Figures 10 and 11). After about 50 days there is negligible further increase in the proportion of soluble nitrogen. Samples stored at +2°C showed a very slow increase in nitrogen solubility even after 12 months of storage.

Backhoff (1976) studied the rate of formation of soluble nitrogen in silages produced from different parts of cod (gut, skin, head and flesh) by the addition of 3% (w/w) formic acid, stored at pH 3.9 and 30°C. He noted that the head showed less proteolytic activity than the skin and gut in the storage period (26 days). The lowest amount of soluble protein was obtained with flesh silage(see Figure 12).

The production of ammonia in acid silage is low because the amino acids are rather stable at low pH.

2. Changes in the oil

During storage of fish silage the characteristics of any fish oil present change. In a commercial operation it would be difficult to remove all of the oil without sophisticated and expensive solvent extraction techniques. Fish oil is best stored in conditions free from moisture and protein particles so that the oil in fish silage is stored under far from ideal conditions.

The free fatty acid content of oils is often used as a criterion for judging the condition and edibility of the fat for commercial purposes. Levels of less than about 4% free fatty acid (as a percentage of total fatty acid) are required for high quality oils.

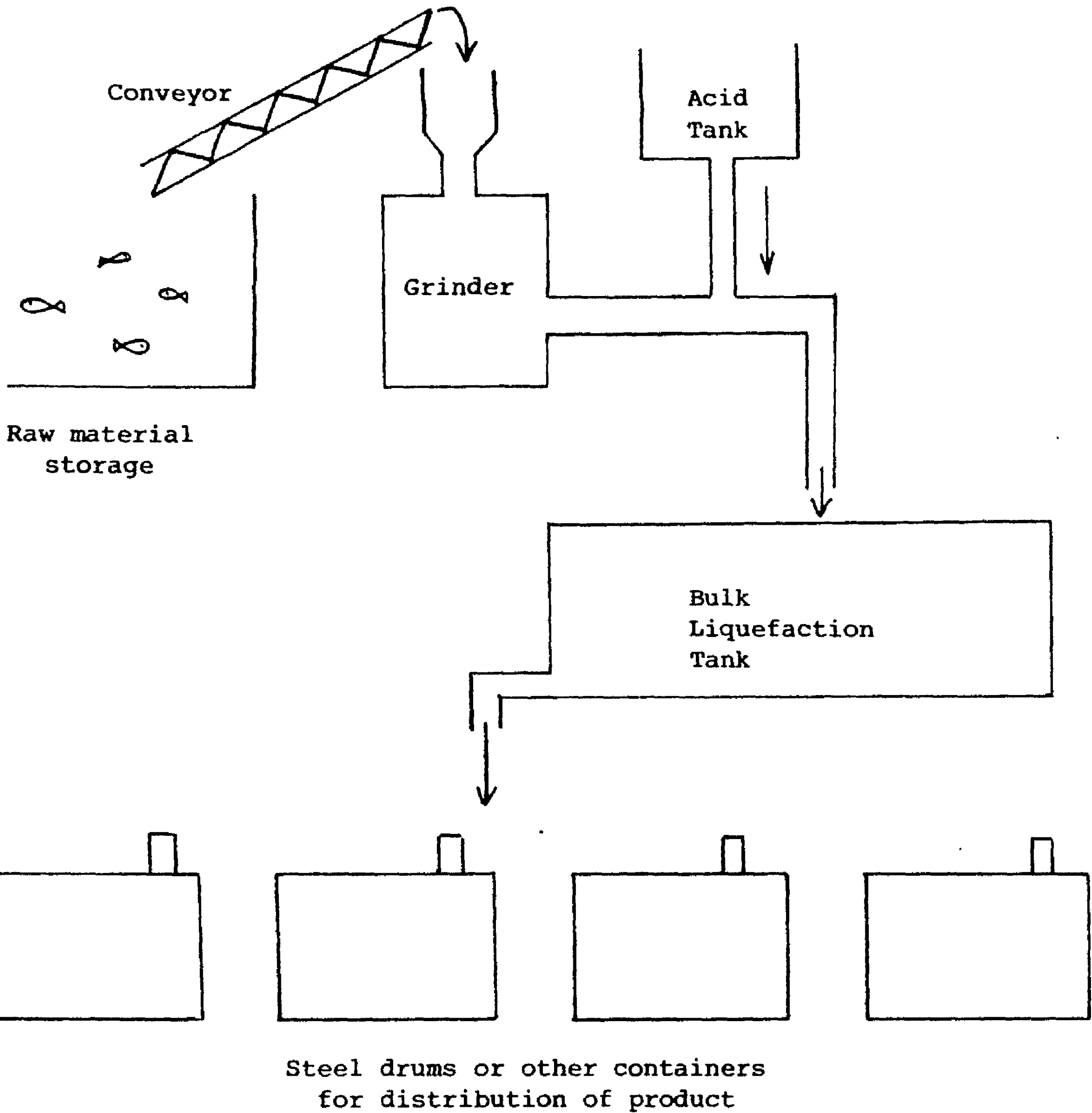
Tatterson and Windsor (1974) investigated the change in oil during a 12 month period for silages prepared from different raw materials. They found that during the storage period the free fatty acid (FFA) content increases, indicating decomposition of glycerides by lipase enzyme. The results for free fatty acid content and Iodine value at the beginning of the storage and after 12 months storage are shown in Table 15..

The free fatty acid (FFA) content of the freshly prepared silages varies from 2.7 to 6.6% of total fatty acid. In all cases there is a very rapid increase during the first 40 days and after 12 months the results show that in some cases more than 20% of fatty acids present in silages stored at 23°C are in the free form. The silages stored at 2°C show a similar initial rate of increase but in general the level reached is somewhat lower. Figure 13 shows the increase of FFA content with time of storage.

Results for iodine value (Table 15) indicate that the values of the oil at the start of storage fall within the range 135 to 175, which is a normal range for such fish oils. In all cases there is a fall in iodine value with mackerel showing the most. At 2°C the decrease in iodine value during storage is smaller than at the higher temperature.

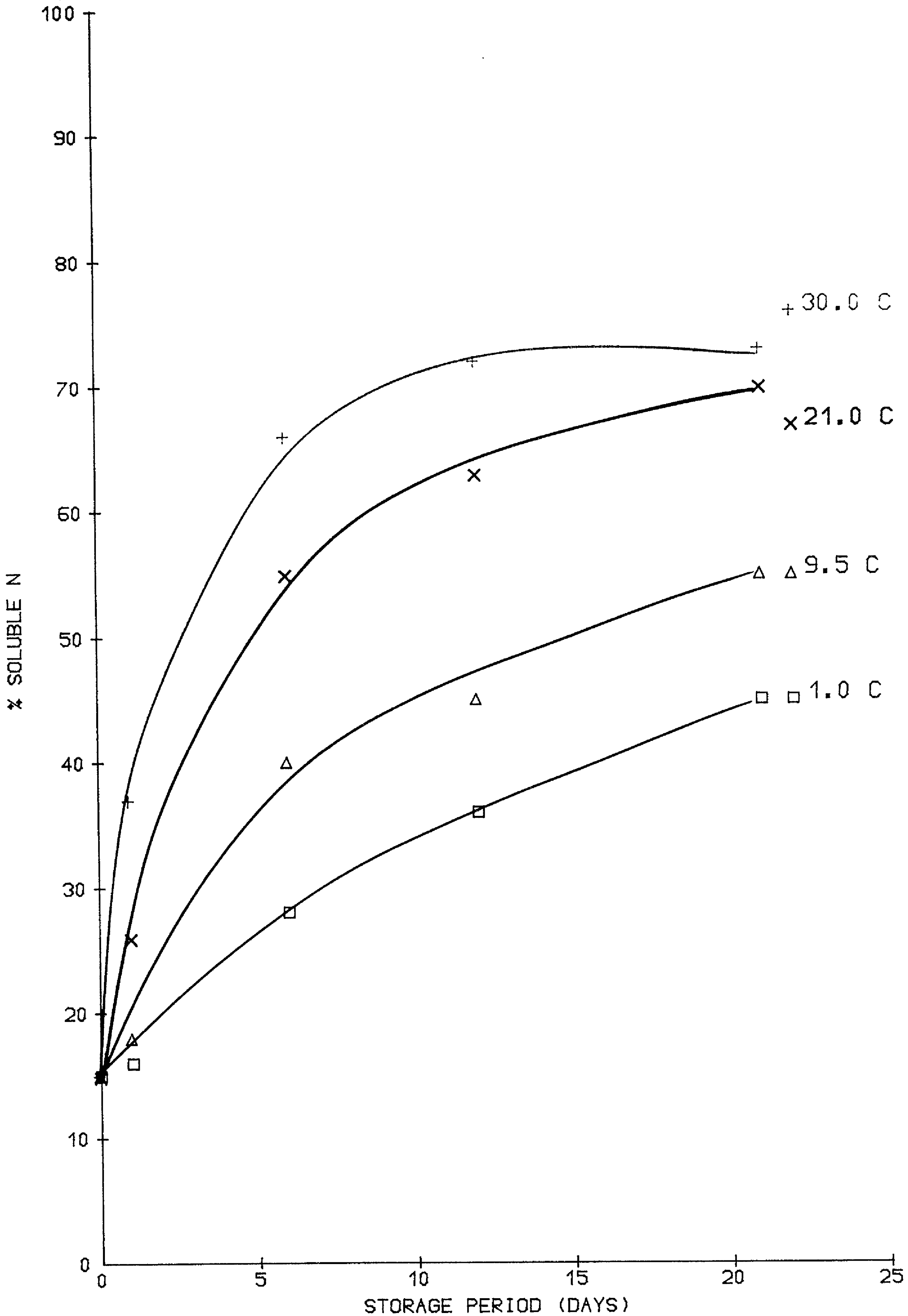
Fish oils are much more stable once they have been separated from fish silage. Figure. 14 shows that a fall in iodine value of extracted oil with time is small and approximately linear with time.

Figure 8: A simple industrial system for fish silage production

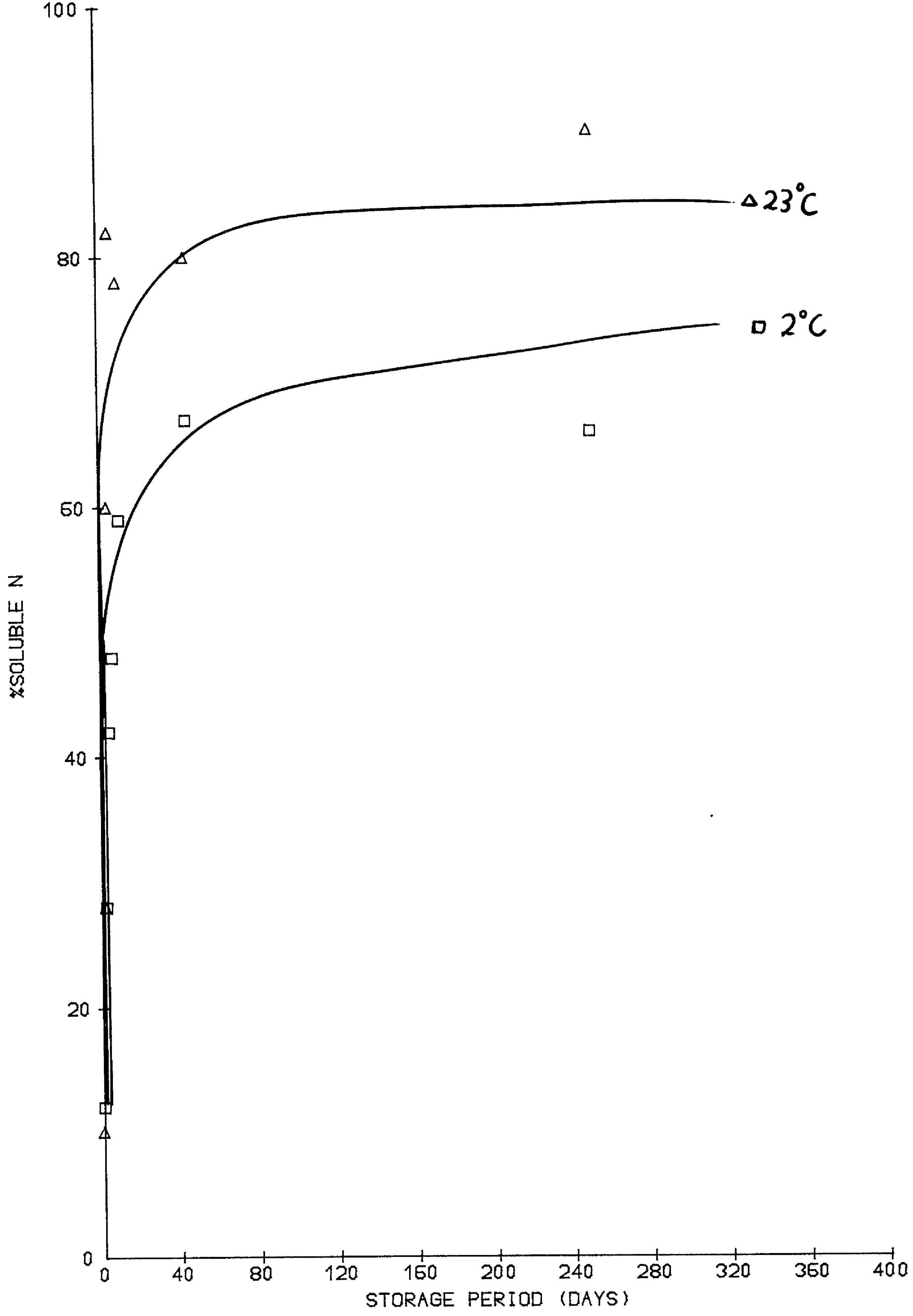


*Tatterson and Windsor (Torry Advisory Note No. 64)

FIGURE 9 . SOLUBLE N AS % TOTAL N IN SPRAT SILAGE STORAGE

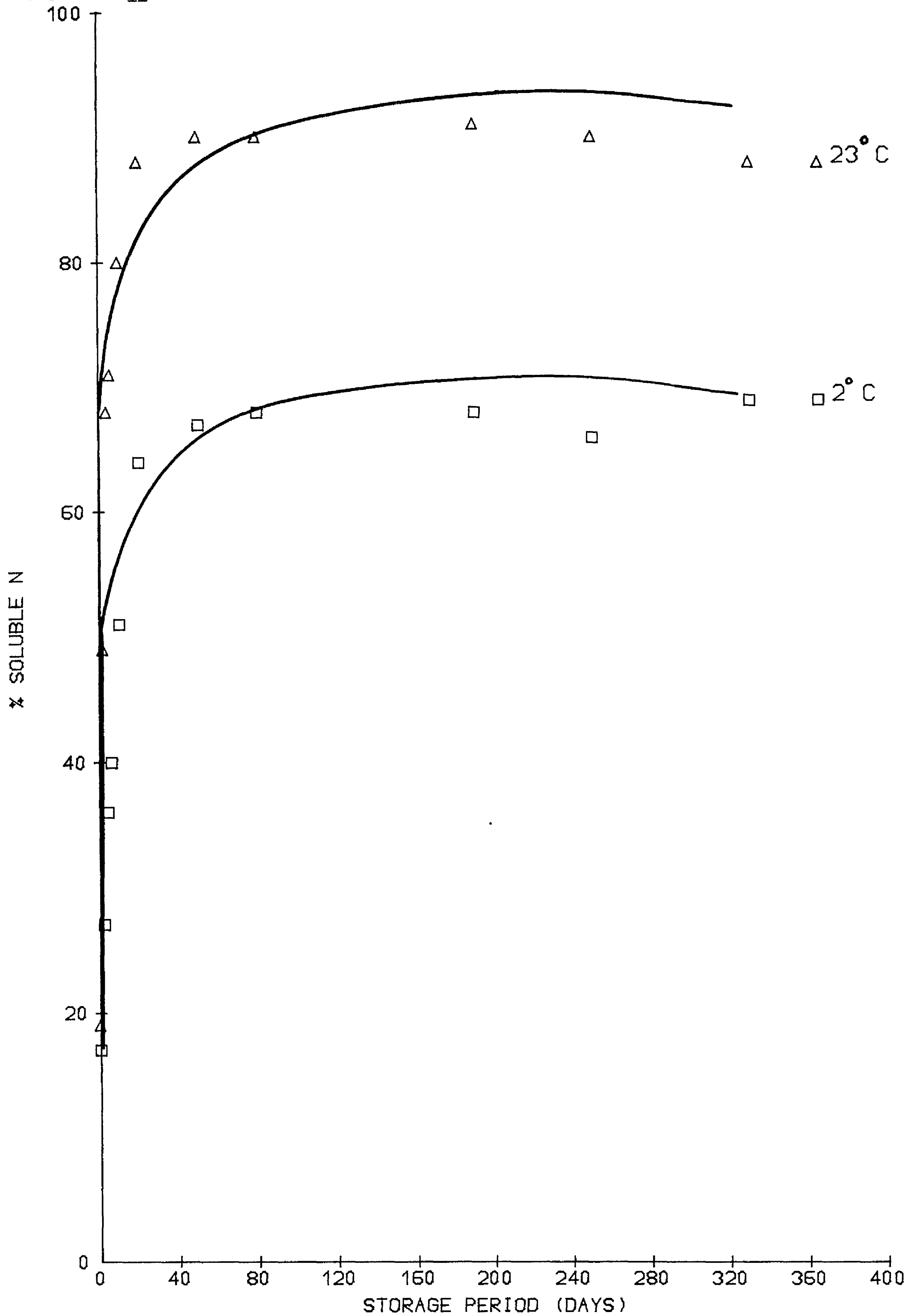


*DISNEY ET AL. (1977)



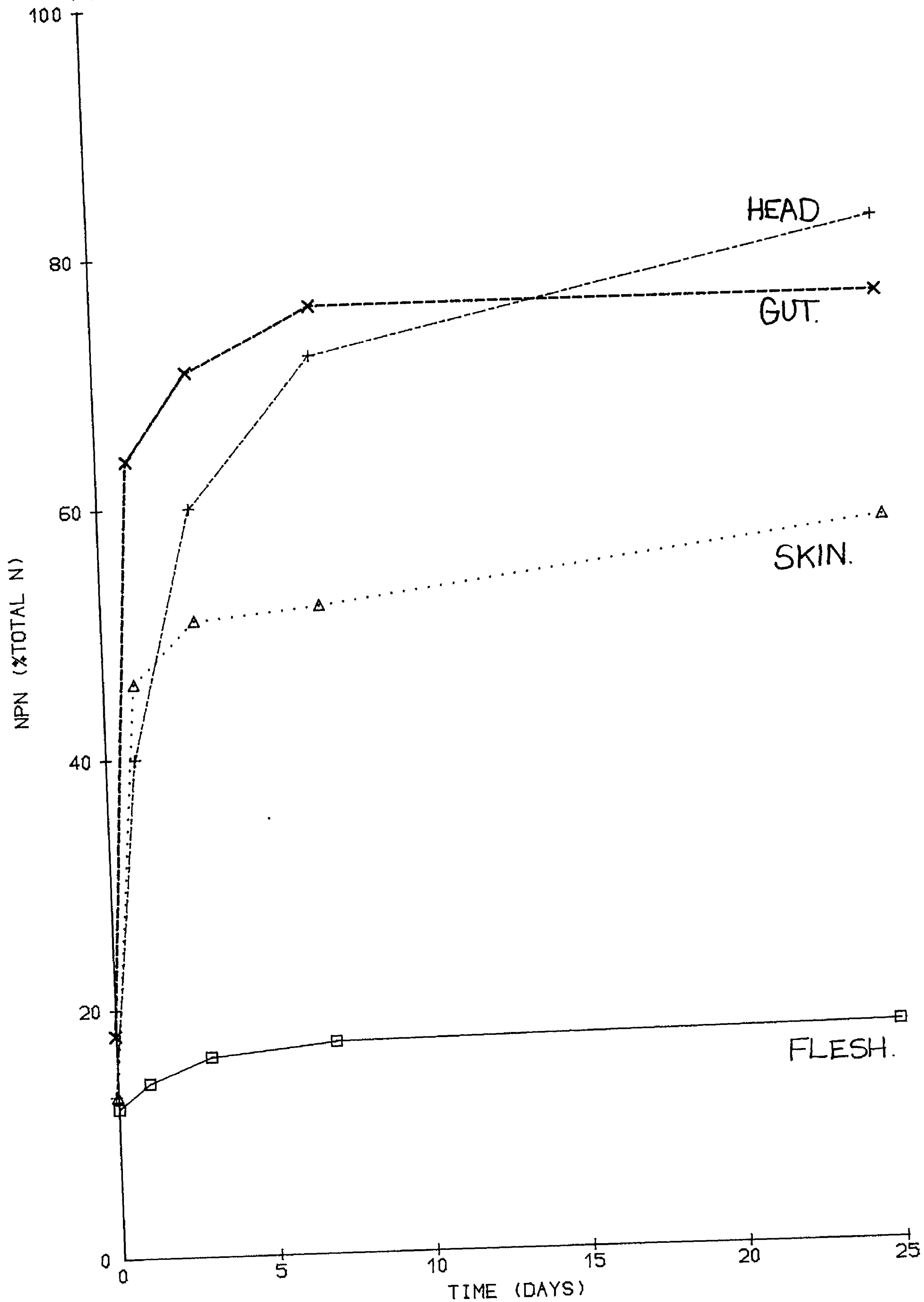
*TATTERSON AND WINDSOR (1974)

FIGURE 11. SOLUBLE N AS % TOTAL N IN STORAGE OF SPRAT SILAGE



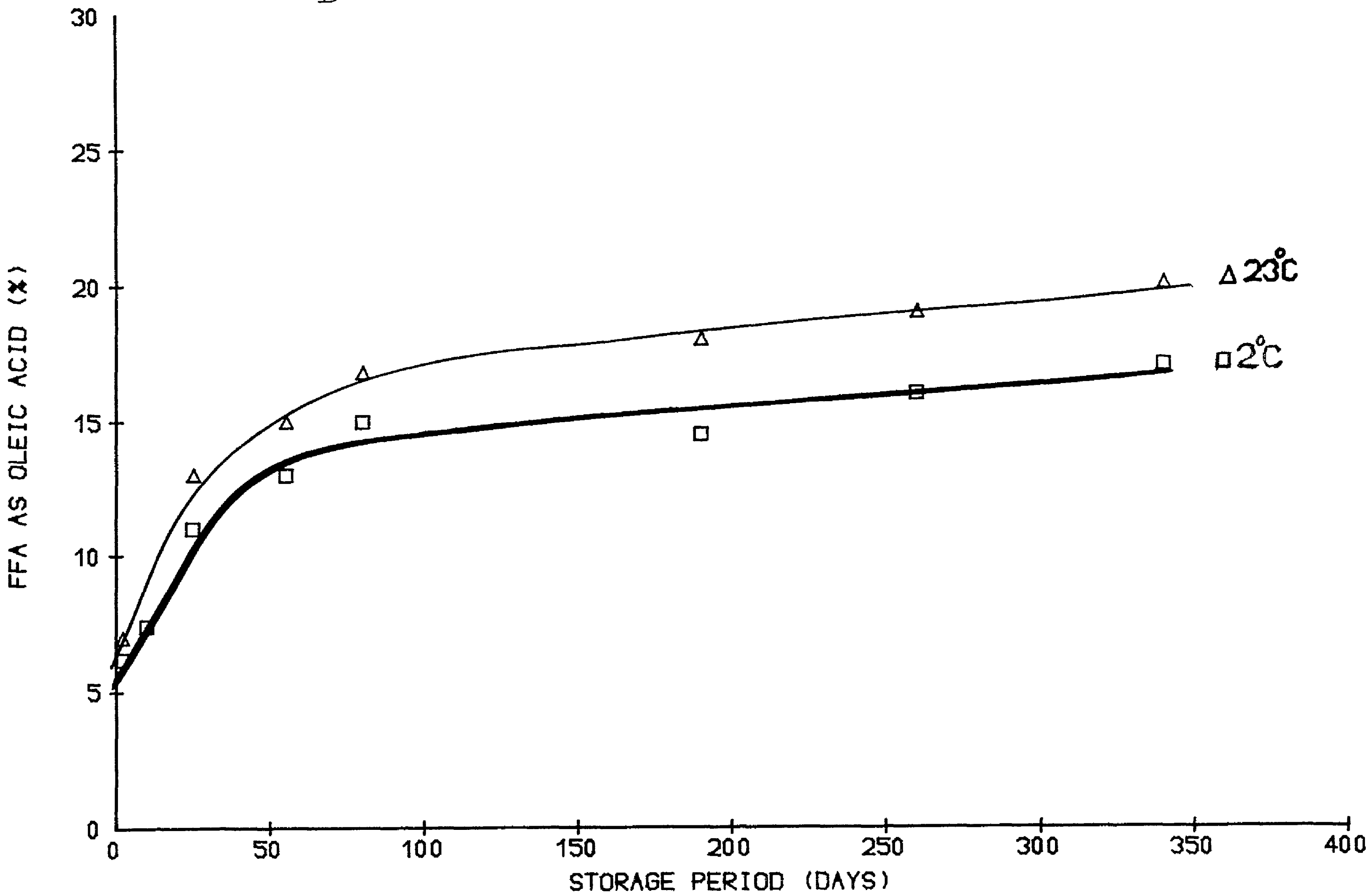
*TATTERSON AND WINDSOR (1974)

FIGURE 12. FORMATION OF NPN IN SILAGES FROM COD PARTS



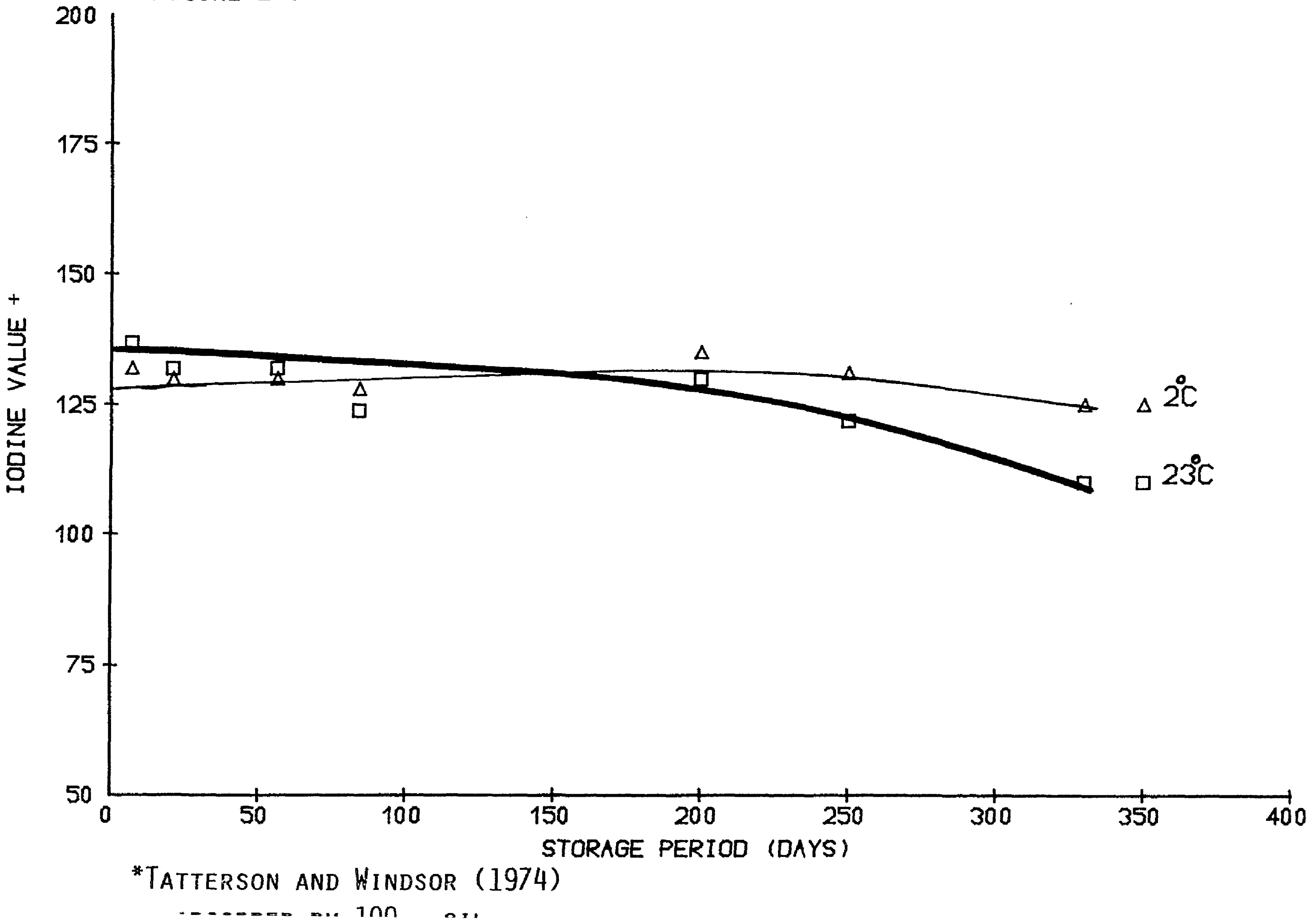
*BACKHOFF (1976)

FIGURE 13 FFA CONTENT OF OIL DURING STORAGE OF SPRAT SILAGE



*TATTERSON AND WINDSOR (1974)

FIGURE 14: IODINE VALUE OF OIL IN SPRAT SILAGE DURING STORAGE



*TATTERSON AND WINDSOR (1974)

TABLE 15

Changes in the oil during storage of fish silage

Analysis	Type of silage									
	Whole sprat		Whole herring		Herring offal		Whole sand-eels		Whole mackerel	
	+2°C	+23°C	+2°C	+23°C	+2°C	+23°C	+2°C	+23°C	+2°C	+23°C
Free fatty acid+ (as oleic acid)										
1. At start	6.6	6.6	5.0	5.0	2.7	2.7	4.2	4.2	6.1	6.1
2. After 12 months	17.4	20.0	18.7	21.4	7.9	12.6	21.5	25.7	12.1	17.2
Iodine value										
1. At start	135	135	147	147	147	147	175	175	163	163
2. After 12 months	123	114	124	104	110	100	94	145	142	108

*Tatterson and Windsor (1974)

+Free fatty acid as % of total fatty acid

Tatterson (1976) also studied the change in oil during storage. The oil examined was extracted from deoiled herring offal silage preserved with formic acid. The FFA content and IV of the oil were measured over a period of 109 days and the results showed an increase of 0.3% in FFA and a fall of 1.4 units in IV, both of which must be regarded as insignificant and extremely low. There did not appear to be any additional deterioration in the colour of the oil during such storage. He suggested that providing oil is separated as soon as possible, fish silage can be stored for a long period without much further deterioration.

The effect of oil oxidation during storage on the nutritive value of acid silage will be discussed later in more detail.

During storage, properly preserved silage becomes smooth in consistency and develops a pleasant malty odour with a brown gravy colour. When fish silage deteriorates, very offensive spoilage odours and obvious signs of fermantation occur. These characteristics are main 'on farm' tests for fish silage quality.

Silage made from fatty fish is more homogenous than that of white fish offal, and there is little separation even after prolonged storage (Tatterson and Windsor, 1974). Therefore occasional agitation will be advisable to prevent sedimentation.

Once the silage is prepared it can be handled like any other liquid. The fish silage can be stored in the processing tank but if more than one batch is to be prepared per day, suitable storage tanks or drums will be required plus a suitable store at the correct temperature.

Equipment requirement

Equipment used for silage production depends on the scale of production. On the small scale it might be sufficient to pulp the raw material, add the acid manually, mix in a suitable container and store in a warm place. For lager scale production however, a mincer capable of reducing material to the required size is essential together with suitable heavy duty mixing equipment to ensure that a homogenous mixture of fish and acid is made. For safety, a pump or measuring device for handling acid is advisable. Suitable tanks

are required for initial liquefaction of the fish, together with other tanks for bulk storage of the finished product and formic acid. Efficient centrifugre and decanter equipment is also required for large scale production to lower the oil level and separate the sediment from the silage. All the equipment involved in acid silage production must be made from acid resistant material.

Composition of fish silage

The proximate composition of fish silage is similar to that of the raw material from which it is made, apart from the slight dilution effect of the acid.

Table 16 shows the proximate analyses of six types of fish silage made using 3% (w/w) of 98% formic acid.

TABLE 16

Proximate analysis of silages made from different materials

Analysis	Types of silage					
	Whole sprat %	Whole herring %	Herring offal %	Whole sand-eels %	White fish offal %	Whole ungutted mackerel %
Crude protein (NX 6.25)	16.7	15.5	14.5	15.4	15.0	16.9
Oil content	6.4	4.4	16.3	3.4	0.5	12.0
Moisture content	74.3	77.7	68.1	77.7	78.9	70.2
Ash content	2.7	2.1	2.6	2.4	4.2	2.1

*Tatterson and Windsor (1974)

The moisture content is high because the product contains all of the moisture from the original raw material. Where the moisture content is high the oil content is correspondingly low. Whereas in oily fish the moisture content is low. Some drying occurred during the period of storage but the extent depends upon the temperature and the length of storage.

A possible quality control would be a dry matter determination. The use of a hydrometer for a quick dry matter determination may have

possibilities. A tendency towards sedimentation has been noted with fish silage. Measurements taken after four weeks show a gradient in dry matter content from the bottom to the top of the liquid, but the material was readily agitated to its original homogenous condition (Hillyer, et al., 1976).

The protein contents of all of the six silages are within the range 14.5 to 17.0%. The four silages made from whole fish contain an average of 16.1% protein, the other two from filleting offal contain an average of 14.8%. Thus due to the relatively high protein content of fish flesh in normal fish frames and heads, the protein content of silages made from such material differs very little from that made from whole fish and were close to the bottom of the range for whole fish.

The ash contents fall within the range 2.1 to 2.7% with the exception of white fish offal silage where the ash content was 4.2.

Oil content was more variable than protein content, ranging from 0.5% in white fish offal silage to 16.3% in the silage of herring offal. There are some variations in oil content, even within the same species caused by seasonal factors. Commercially it would be desirable in any large-scale operation to remove the oil from silage if it exceeded about 2 to 4% (Tatterson and Windsor, 1974).

Table 17 shows the composition of silage fractions after the removal of oil.

TABLE 17

Composition of silage fractions

Fraction/Component	Moisture %	Ash %	Oil %	Apparent protein (N x 6.25) %
Decanted solids	61.7	10.2	1.6	24.0
De-oiled silage	81.1	2.6	1.5	13.8
Desludge	76.3	2.0	7.8	14.4

*Wignall and Tatterson (1976)

The silage was made using waste from herring filleting using formic acid as a preservative. Both decanted solids and de-oiled silage contain little oil and the percentage of protein in the decanted solids is almost twice that in the de-oiled material. A mixture of these two fractions contains 14.9% protein. Desludge contains about the same percentage of protein as de-oiled silage, but has a much higher oil content.

If this fraction were to be added to the mixture of de-oiled silage and decanted solids, the final mixture would contain almost 2.5% oil, which is too high for the product to be used as a source of animal feed. Desludge could perhaps be used by blending it with a silage made from white fish offal and hence containing little oil; this could increase the nutritional value of the latter.

Table 18 shows a comparison between the composition of fish silage and fish meal, both made from white fish, but not from the same batch.

TABLE 18

Proximate composition of white fish silage and white fish meal

Composition	Fish silage	White fish meal
Dry matter %	21.2	90
Composition of dry matter		
Crude protein %	70.3	70.1
Ether extract %	3.1	4.0
Total ash %	16.4	24.1
Calcium %	3.8	7.9
Phosphorus %	11.9	4.3
Calculated digestible energy MJ kg^{-1} DM	14.4	13.1

*Smith and Adamson (1976)

The protein and ether extract levels are similar, but the total ash, calcium and phosphorus levels in the white fish meal are higher than fish silage. The energy value of fish meal is lower than fish silage. However, the fish meal energy value reported above may be low because Whittemore and Taylor (1976) found in their experiment that the energy value was 15.7 MJ Kg^{-1}DM using the same digestibility coefficient.

Benefits of fish silage

Several problems are likely to be encountered in the production of fish meal. The principle problem is the supply of raw material characterised by scattered and irregular landing of fish, or when the fish quantities are too small for viable operation of a fish meal plant, but other problem areas are packaging, marketing and storage. In developing countries imported packaging materials are expensive. There is also a shortage of shipping space in some areas and the product may have to be stored for long periods under unfavourable tropical conditions. Furthermore, unless the fish meal is kept in a well-designed store, insect infestation is likely to cause serious problems.

In such situations an enormous amount of surplus fish and fish waste are often underutilised. Therefore fish silage may become a competitive process and provides a means of utilising fish that would otherwise be wasted.

The main advantages of fish silage over fishmeal are that:

1. The capital investment required is considerably less than that required for fish meal plant.

2. The major problem of smell associated with fish meal production is not a problem with fish silage.

3. Fish silage plant is relatively simple to operate and requires a minimum of labour, skilled or otherwise.

4. No energy is required to heat the product with many types of fish silage, a major factor with the increasing cost of energy.

5. Acid preserved fish silage does not putrefy, retaining a fresh acidic smell even after storage for weeks.

6. The scale of production of fish silage can be varied at will without economy of the process being much affected.

7. A fish silage is almost sterile and pathogens like Salmonella are efficiently killed in it. Fish meal has spread this pathogen world wide.

8. Economic studies carried out in Sri Lanka, Mexico and the UK all concluded that fish silage production was a viable alternative to fish meal.

9. Research on the use of fish silage as a component of animal feed has given good results without complications.

The disadvantages associated with fish silage:

1. The economics of distributing silage with its large water content, four to five times as bulky as fish meal, restrict the product from being distributed on a 'national' scale.

2. There are nutritional problems to be resolved of acid-preserved fish silage, particularly the poor growth and palatability when fed to poultry.

3. This new product will require promotion for it to be accepted as an animal feedstuff, while fish meal is a long-established and well known product.

4. When liquid silage is stored for a long period of time, mould growth occurs at the surface and mycotoxins (i.e. a flatoxin) could be produced.

5. The degradation of vitamin B_1 (Thiamin) which is quickly decomposed in moist feeds containing fish with thiaminase activity and the need for supplementation.

6. The fish taint in the animal reared on fish silage with a high oil content which is unacceptable to most consumer groups. The fishy taint can, however, be metabolically removed during the last weeks before slaughtering by feeding rations without fish lipids, but rich in plant oils.

7. The autolytic degradation of fish protein during ensilage may restrict its value as a source of rumen undegraded protein (UDP) for ruminant stock.

Despite these disadvantages an investment in silage could be profitable to an area with no outlet for waste fish or offal, given the right circumstances of continuity in raw material supplies and farm location.

Nutritional value of fish silage

Acid preserved fish silage is, like other food of fish origin, very valuable as a source of protein and vitamins. When fish silage has been produced from fish of low oil content, it can be used as a direct replacement in the diet of pigs and poultry for fish meal on the basis of equivalent dry matter.

Fish meal contains about 65% protein whereas fish silage contains about 15% (on a fresh weight basis) so that about four times as much silage is required for the same protein intake.

As a simple guide 4.5 kg of fish silage from low oil fish is equivalent to 1 kg of white fish meal. If the silage has been made from whole oily fish such as herring or mackerel then the rate of feeding it should be limited (Anon, BP Nutrition, UK). If the fish silage adds more than 1% of fish oil to the whole ration then the palatability of the ration will be reduced, and meat or egg taints may be caused.

Feeding trials and feeding experiments with pigs poultry, cattle, mink and fish have been carried out and indicate that acid-preserved fish silage is a good source of protein and that its nutritional value is, in many cases, comparable to that of fish meal.

However, some reports show that acid fish silage is inferior to fish meal, in particular in poultry diets.

Feeding trials with runimant species have been limited compared with work with monogastric species. The indications are that fish silage, although a valuable protein source, may not be as effective as traditional protein supplements.

(a) Feeding trials with pigs

The satisfactory use of acid fish silage in pig feeds has been well demonstrated by a number of workers.

The Danish National Research Institute on Animal Husbandry (Denmark, Landøkonomish-Forsøgslaboratorium, 1952) carried out an experiment with pigs, in which milk was substituted by fish meal or fish silage

(made from the same raw material - herring with a content of 1.7% fat) in the live weight, range of 30 to 50 kg. In the experiment four different sorts of fish silages were tested:

1. formic acid;
2. sulphuric acid/formic acid;
3. sulphuric acid/molases;
4. fermentation.

The results of the experiment proved that feed of fish origin has at least the same value as skimmed milk as a source of protein. However, fish silage no matter what preservative was employed, should only be fed to pigs of more than 50 kg live weight to avoid deterioration of the flavour and keeping quality of the bacon. To be on the safe side they suggested that only low levels of fish silage should be fed to pigs under 30 kg live weight.

This restriction would be unnecessary if de-oiled silage was used. Jensen (1973) in Denmark found that silage prepared from whole fish preserved with formic acid alone, or 5% sulphuric acid plus 1% formic acid, to be nutritionally acceptable when fed to pigs.

Cameron (1962) reported equal feed conversion efficiencies and daily live weight gains for pigs fed on a cereal diet supplemented with either acid fish silage or fish meal.

Disney et al. (1978) found that a formic acid silage of tuna offal was slightly better than a fish meal diet. No adverse effect on carcass quality was noted, even though the fish lipid level in their silage diet was 1.1% of dry weight. The results of Batterham and Groman (1980) also indicated that pigs fed on a cereal diet with fish silage produced a live weight gain significantly better than on a diet based on soya protein.

In the United Kingdom, silage prepared from white fish offal, containing 70.3% protein and 3% ether extract on a dry matter basis, has been fed at the 10% dry matter inclusion level to bacon pigs. Weight gains were as good as those found in pigs fed an equivalent diet containing conventional non-cereal protein (white fish meal and

soya) were found, and good quality carcasses, free of taint, were produced. Equally important has been an attempt to find the maximum safe level of fish oil in the diet to avoid tainting of the carcass. Trials already completed with a silage made from herring offal, containing 42.7% protein and 43.9% ether extract on a dry matter basis, have shown that taint occurred at a dry matter inclusion level of 5% (13.7% of the dry matter of the diet) but not when 2.4% was used (Smith and Adamson, 1976).

Hillyer et al. (1976) carried out a feeding trial to compare de-oiled acidified herring silage with commercial soya bean meal as a supplement to barley based diet at two lysine levels. The pigs were grown from 30 to 60 kg live weight. They found that growth rates and feed conversion of pigs fed fish silage diets were significantly higher than the control diet. None of the carcasses of pigs fed fish silage or control diets were down graded by (MLC) classification. Farmers using Danish fish silage have claimed that it improved the fertility of animals. This suggestion has experimental support (Lisac, 1961). On the other hand these are reports on the slightly inferior nutritional quality of fish silage as a pig feed. This has been observed when the inclusion rate of silage was as high as 25% of the dry matter of a complete diet (Whittemore and Taylor, 1976).

Smith and Adamson (1976) reported similar results even at an inclusion rate of 5-10% of the dry weight of a barley based diet, which is a reàlistic level for practical feed formulation. Relatively poor weight gain has been obtained also with pigs fed on fish silage absorbed on barley (Sumner, 1978). However, the difference in performance between the fish silage rations and the control would be outweighed in a normal farm situation by the savings that are possible in feed costs. Therefore, the difference in performance would hardly be noticed.

The greatest restriction to the use of fish silage in rations for bacon pigs is the fishy off-flavour it may cause in the carcasses. However, there are no indications that this is a more severe problem with fish silage than it is with a fish meal having the same oil content. In order to avoid carcass taint, it is recommended that the level of fish oil in the final diet be kept below 1% of the dry

weight, and below 0.6% during the last days of feeding (Barlow and Pike, 1977). An upper limit of 1% seems to be valid also when the oil derives from fish silage (Smith and Adamson, 1976; Disney *et al.*, 1978). However, if silage based diets have been fed, it is advisable to give conventional meal the last day or two before slaughter. Otherwise the gut contents acquire a fish odour, which may be so disgusting for the slaughtermen that they may refuse to carry out the work (Sumner, 1978).

Successful use of silage in pig diets requires control of the silage oil content by removing the oil after autolysis and careful manipulation of dietary inclusion level.

There may be difficulties in mixing liquid silage with dry meal and feeding by means of self-feeders.

Alternatively the silage can be offered as a free choice in addition to the dry meal diet, or fish silage (alone or with water) could be distributed separately into troughs allowing the pigs to mix the diet during normal eating. This latter system has worked satisfactorily in feeding trials carried out with pigs (Hillyer *et al.*, 1976).

(b) Poultry feeding trials

The first feeding experiment with fish silage diets, by Edin before 1940, showed that herring silage preserved with sulphuric acid was an excellent feed constituent in the rations for chickens and layers. Growth rate and overall performance were the same as on diets composed of other protein sources. Moreover, the silage contributed sufficient vitamin D. The inclusion level of neutralised silage corresponded to 30% of the total protein in the diet for chicken and 12 to 23% for broilers. At the former inclusion level which gave an oil content of 2.7%, the carcasses had a fishy taint, but the eggs were of good quality. This is in accordance with later experience, that fish oils can be included in the diet for layers without tainting the eggs (Hansen, 1959).

Rattagoal (cited in Kompiang, Darwanto and Arifuddin, 1980) found that the nutritional value of fish silage prepared by an addition

of 3% of 1:0.5:2 mixture of formic acid, pripionic acid and sulphuric acid was similar to fish meal. In Sri Lanka, Poulter et al. (1980) reported good weight gains and feed conversion efficiencies in broiler starters and finishers fed diets containing dried fish silage product prepared by adding maize meal to sardine silage produced with formic acid. The level of silage in the diet was 20%.

On the other hand some feeding trials have shown unsatisfactory animal performance, particularly when fish silage is included at high levels in poultry diets (Disney et al., 1978; Disney and Hoffman, 1976; Kompiange, Arifudin and Raa, 1980; Poulter et al., 1980). It seems clear that acid fish silage contains some factors which retard the growth of poultry, particularly when fed at relatively high levels (Disney et al., 1978). The following factors have been considered as possible reasons for the disappointing animal performances.

1. Residual organic acid

An apparent explanation for the low acceptability of silage feed is the residual acid. This is particularly so for silage-based dry chicken feeds which contain a high residual concentration of the preservative acids, even when relatively volatile organic acids are used. However, Disney and Hoffman (1976) reported that chicks preferred a commercial dry diet containing 1% formic acid. This has also been confirmed with propionic acid (Kompiang, Arifudin and Raa, 1980).

2. Thiamine deficiency

Since most fish contains an enzyme which degrades thiamine (vitamin B_1) there is a certain risk that feeding fish silage rations may cause a deficiency in this vitamin. Poor growth on silage diets has been attributed to vitamin B_1 deficiency (Disney et al., 1978; Disney and Hoffman, 1976). Heating the silage is a means of avoiding this risk, by inactivating any thiaminase in the silage. However, thiamine injection did not improve the general performance of the chick (cited in Kompiang, Arifudin and Raa, 1979) which suggests that thiamine deficiency may not have been a problem.

3. Degradation of essential amino acids

Most amino acids are stable in fish silage. Only 1.3% of the amino nitrogen was released as ammonia in a silage of by-catch fish after three weeks at tropical temperature (Kompiang, Arifudin and Raa, 1980). Nevertheless, this might imply a significant reduction of the nutritive value of the silage if the ammonia was derived from a few essential amino acids.

Tryptophan and histidine are unstable when they are free, but stable when bound in proteins. Free tryptophan decomposes in an acid silage (Backhoff, 1976; Kompiang, Arifudin and Raa, 1980) and there are reports claiming that methionine (Atkinson et al., 1974) and histidine (Disney, et al., 1978) are also unstable.

Therefore, if the degree of autolysis is high, or if the silage is desludged, diets with a high inclusion level of fish silage may have a deficit in these amino acids.

After storage for 40 days at 30°C about 30% of the tryptophan was lost in silage of cod and herring preserved with formic acid at a pH slightly below 4 (Backhoff, 1976). At lower storage temperatures the rate of degradation was significantly lower. Whether enzymes are involved in the degradation of tryptophan is not known, but there are observations in favour of this suggestion: for example, tryptophan was shown to be stable in the heat-treated aqueous phase of a silage made from cod or saithe viscera (Strøm and Eggum, 1981).

Although a significant reduction in the level of tryptophan was detected after 21 days at 30°C in an acid silage of by-catch fish, this reduction could not account for the markedly lower feed conversion efficiency of diets based on this silage compared to diets based on fish meal (Kompiang, Arifudin and Raa, 1980).

It has been reported that methionine was the growth limiting amino acid in fish silage (Jensen and Schmidtsdorff, 1977), but this was probably due to a low level of this amino acid in the raw material. Histidine may be the limiting amino acid in fish silage (Disney et al., 1978) particularly if the silage is prepared from partly spoiled fish, since this amino acid is quickly degraded by spoilage bacteria at neutral pH.

4. Partial spoilage of raw material

The rate of spoilage under tropical conditions is so fast that a significant reduction in nutritional value of by-catch fish can be recorded already after 12 hr (Disney and Hoffman, 1976).

To examine whether partial spoilalge of the raw material could account for the poor feeding values, silage was prepared on board the trawler from completely fresh fish. Nevertheless, the nutritional value for poultry was still poor (50 to 60% live weight gain) compared with fish meal produced from the same raw material.

5. Toxic products in the lipid fraction

Unsaturated fatty acids in lipids react with oxygen and form hydroperoxides as the initial products. Lipid hydroperoxides may be quite stable in organic solvents or in the lipid phase itself, but in foods they decompose readily through a free radical chain reaction into stable secondary products (Dugan, 1975). The level of lipid hydroperoxides in foods attains a maximum during storage. This maximum may be reached in a few days or months, depending on many factors including, temperature of storage, availabe oxygen, the presence of pro- and anti-oxidants, and reactivity of the lipids.

The concentration of hydroperoxides in fat is usually determined by the iodometric method (Lea, 1946) and the peroxide value is expressed as milli-equivalents of peroxide per kilogram fat (Ockerman, 1978).

The peroxide value is a useful analytical figure, since oxidized fat in animal rations causes loss of appetite, decreasing live weight gain and even death if the peroxide value exceed 100 m.eq. per kilogram of diet (Dugan, 1975).

The typical off-flavours (rancidity) of oxidised fat are attributed to volatile carbonyls formed during decomposition of hydroperoxides (Labuza, 1971). These secondary products of lipid oxidation may react with proteins and thus cause some reduction of the nutritional value (Gardner, 1978). The oxidised products may also be deposited in animals fed on a rancid diet and thus be responsible for carcass taint. However, protection from oxidation by the use of antioxidants may sometimes cause more carcass taint, because the stabilised polyunsaturated fatty acids themselves are deposited in

the animal and give taint to the carcass (Opstvedt et al., 1971).

There is little information on the peroxide value of fish silage or silage-based moist feeds during storage. Disney et al. (1978) reported that the peroxide value did not decrease during one month of storage of such feed at tropical temperatures, and was then 132 m.eq/kg fat. After further storage for two months this value fell to about one-third. However, a good chicken feed could be made from a fish silage that had been extracted with ether before it was mixed with corn and sun dried (Kompiange, Arifudin and Raa, 1980). The same authors suggested that boiling of the silage, prior to mixing with corn, improved the feed conversion efficiency of the silage diet. There was no difference between performance of the chickens fed the silage diet that was boiled immediately after acid addition and that boiled after ten days of autolysis. This suggests that enzymatic autolysis is of minor importance in the deterioration of the nutritional value of the silage. It appears that boiling either removes a component in the raw material that impairs the chickens ability to utilise the silage diet or generates inhibitors of chemical transformations which yield toxic products in the dried feed.

However, even when chickens were fed de-oiled silage, slightly lower feed conversion efficiency was found, compared to a fish meal diet, suggesting that factors in the oil fraction are not the entire cause of the problem.

6. High levels of free amino acids

Fish silage contains a high level of free amino acids, which may have a lower nutritional value than proteins with the same composition possibly because high levels of free amino acids may saturate absorption mechanisms.

This possibility does not explain the poor nutritional value, since inactivation of the autolytic enzymes by boiling prior to ensiling for ten days yielded a silage with the same nutritional value as that left to autolyse for ten days before it was boiled (Kompiang, Arifudin and Raa, 1980).

7. Manganese deficiency

Perosis/slipped tendon may be a symptom of manganese deficiency in poultry and has been observed in chicks fed fish silage diets (Poulter et al., 1980). It was shown, however, that silage fed birds contained the same manganese level in the tibia as birds fed on fish meal.

8. Vitamin E deficiency

The apparent toxic effect of silage diets may be indirect, through decomposition of vitamin E and Vitamin A by oxidised lipids. The growth response of chickens fed on a silage/cassava diet was improved when vitamin E was supplemented. However, in other cases, vitamin E had no significant effect (Kompiang, Arifudin and Raa, 1980).

Further work should be carried out on the feeding silage products to poultry. Topics to be investigated include storage of the liquid silage and the dried product, the presence of toxic amines, the effect of lipid oxidation (including the use of anti-oxidants), thiaminase activity and vitamin supplementation and a comparison of fresh and spoiled fish.

(c) Fish feeding trials

Silage based moist pellets have been shown to be an excellent diet for salmonoid fish in Norwegian fish farms. However, salmon does not tolerate propionic acid among the preservative acids, and differs in this respect from trout (Austreng, 1982). A characterist softening of the gut has been noted though, but this has no apparent effect on the general performance of the fish.

Djajasewaka and Djajadiredja (1980) observed that freshwater carp also accept and grow well on silage-based feeds. They found no significant difference in body weight gain between fish meal, boiled fish silage and raw fish silage. The body weight gain of fish fed raw or boiled silage was slightly lower than those fed fish meal. This might be partly due to the lack of vitamin B_1 judged by the appearance and behaviour of the fish, and may in part be due to thiaminase possibly present in the silage.

(d) Ruminant feeding trials

Due to the high proportion of cattle in northern Norway, most of the fish silage feed is utilised by these animals. Results from the feeding experiments of the Norwegian University of Agriculture indicated practical daily intakes of 4 kg and 1 kg to cattle and sheep respectively (Strøm, et al., 1979).

De-oiled silage of cod and saithe viscera are produced commercially in Norway and distributed there for animal feeding (Schantz, 1974). A mixture of 55% (w/w) de-oiled and heated silage of viscera, 20% (w/w) minced fish heads, 15% (w/w) barley meal, and 10% (w/w) grass meal has been used for years in practical feeding programmes and the results seem acceptable. This feed, however, gives a slightly lower yield of milk than the commercial high protein meal when fed at a high rate to cows (Johnsen, 1980).

One explanation for this being that ingestion of a high level of free amino acids leads to enhanced microbial ammonia production in the rumen. With monogastric animals this does not appear to be a problem.

In the UK Young and Dunn (1975) have carried out a trial in which weaned calves, fed indoors with hay, straw and bruised oats, were compared with groups where the feed also included a mineral/vitamin supplement and either commercial protein pellets or fish silage. At the end of the indoor feeding period, animals fed with the protein supplemented diets had gained most weight and were in better condition that those fed only the standard farm diet. Furthermore, supplementation with fish silage promoted superior live weight gain compared to the protein pellets. The economic importance of this was shown at the end of the subsequent summer grazing period.

The majority of animals fed fish-silage had fattened sufficiently for sale whilst only about 50% of those fed protein pellets had reached the same stage. The majority of animals in the normal farm feed group required further feeding before reaching the fat stage. The silage was easily absorbed by the fodder and grain, and the animals accepted the mixture readily.

Lambs have been reared successfully from 1 to 2 days old on a

synthetic milk composed of silage of white fish (cod fillets), lard, coconut oil, and lactose. The lambs grew more slowly, however, on this milk replacer during the first 15 days than on a dried skim milk diet, though later growth was equally good (Soliman and Ørskov,1976). Strøm (*) reported excellent nutritional value in liquid fish silage when included in milk replacers for calves. However, commercial enzymatic degradation of fish tissues to increase solubility has been shown to generate bitter tasting peptides which may reduce the palatability of the milk replacer.

(e) Other animal feeding trials

Feeding trials have also been carried out with mink, fox and rats. Skrede (1981) conducted a trial using de-oiled fish viscera silage as a protein source in mink diets. He found no indication of reduced fur quality by feeding fish viscera silage. Reproductive performance was very satisfactory with fish viscera silage diets. He suggested that fish viscera silage are a valuable protein resource for mink production. However, acid preservation would set a limit to its inclusion level in mink diets, because of the sensitivity of the mink to the preservative acids.

Rat feeding tests show that the biological value of liquid fish silage is similar to that of skimmed milk powder (Krishnaswamy *et al*., 1965).

Another feeding experiment with rats has revealed that storage for 60 days at 15°C had no adverse effect on the NPU value of heated and a de-oiled silage of fish viscera (Strøm and Eggum, 1981).

*Strøm, T., Institute of Fishery Technology Research, internal reports Norway

Conclusion/Nutritive value

There is no doubt that acid fish silage is a realistic alternative to fish meal in utilising fish waste, surplus fish and low value fish. The chemical composition suggests that the product has a relatively high nutritive value. Feeding trials also have shown that the inclusion of acid fish silage in animal diets will give satisfactory milk yield, live weight gain and feed conversion eficiencies. The slight depression in performance when fish silage is included may not be of much commercial significance. However, there is still need for further work to investigate factors which lower the nutritive value, including:

1. The effect of protein degradation.
2. Storage of acid fish silage.
3. Lipid oxidation and the use of anti-oxidants.
4. Vitamin deficiency and vitamin supplementation.
5. The presence of toxic amines, e.g. histamine.
6. The effect of spoilage bacteria on the raw material.

   (Comparison of fresh and spoiled fish.)

II - Fermented Fish Silage

The successful preservation of the fish by biological fermentation method is dependent on the production of lactic acid (von Hofsten and Wirahadikusumah, 1972). On media containing sugars, lactic acid bacteria produces large amounts of lactic acid which decreases the pH and thus renders the medium unsuitable for the growth of most other micro-organisms. In addition to the acid, some lactic acid bacteria produce antibiotics (Schrøden, et al., 1980). These bacteria not only prevent microbial spoilage of feeds, but also add flavour to the end product and may prevent rancidification and other chemical reactions which cause quality reduction (Roa, 1965). Fish is rich in protein and lipids but has a very low level of free sugars (ribose, glucose, fructose) availabe for fermentation by bacteria. The most significant source of energy of growth of bacteria in fish is the pool of free acids, which increases as a result of proteolysis of the fish post mortem.

Work carried out in Sweden, by Nilsson and his colleagues, has shown that the addition of suitable carbohydrates improves fermentation. The addition of mixtures of malt and cereal meal, and molasses and tapioca meal have all proved successful (Nilsson and Rydin, 1963; Nilsson, 1969).

The fermentation process for the conversion of carbohydrates to lactic acid is anaerobic and can be divided into three stages (Wirahadikusamah, 1968). Initially the starch of the carbohydrate source is hydrolysed to malt by α or ß amylase. The maltose molecules are then further broken down to glucose by maltase. The last step in the fermentation is the conversion of glucose to lactic acid by lactic acid bacteria. Small amounts of other substances, such as acetic acid and alcohol, are also formed.

Lactic acid bacteria can be divided into two groups, the homofermentative group and heterofermentative group (Hardy et al., 1971). The homofermentative lactic acid bacteria produce two moles of lactic acid per mole of glucose while the heterofermentative bacteria produce only one mole of lactic acid together with ethyl alcohol and carbon dioxide. Therefore, for a successful fish silage fermentation, a predominance of homofermentative strains appears to be

desirable. Although lactic acid bacteria are widespread in nature, they are not found in large numbers in fish microflora. It is, therefore, essential to add a starter culture of lactobacilli for a successful fermentation.

The rate and degree of fish protein degradation in fermented silage has not been examined in as much detail as for acid preserved fish silage. However, it appears that the degree of protein breakdown to soluble components is significantly lower than for acid silage (James et al., 1977).

Explanations include the following:

(i) inhibition of protein breakdown by absorption of enzymes or substrates onto polysaccharides;

(ii) failure of the relatively slow reduction in pH to activate the autolytic fish proteases.

For more information about fermented fish silage see (Petersen, 1953; Roa, 1965; Kirshnaswamy et al., 1965; James et al., 1977; Kompiang Yushadi and Greswell, 1980; Kompiang, Darwanto and Arifudin, 1980; Raa, 1981).

Preparation

Production of fermented fish silage is a simple, cheap method of preserving fish waste. The procedure involves three stages.

(a) Chopping or mincing the raw material (fish waste).

(b) Blending the comminuted material with carbohydrate source (e.g. molases, rice bran, starch) and lactic acid bacteria starter.

(c) Fermentation and storage at a suitable temperature of approximately 30°C.

Nutritional value

Feeding experiments with fermented silage, mainly with poultry, showed that the nutritional quality is good. Krishnaswamy et al., (1965) prepared fish silage from fresh water fish to which lactose,

citric acid, yeast extract and <u>streptococcus</u> <u>lactic</u> were added. The dried silage was found to have a comparable biological value to skimmed milk powder when fed to rats. Nilsson and Rydin (1963) refer to poultry feeding trials using silage prepared from a mixture of malt, cereal and minced fish by the action of lactobacilli. The health of the birds and the production of eggs were good and carcasses were of good quality, lacking any unpleasant taste or flavour.

The production of ammonia is considerably higher than in an acid silage - especially during the first days of storage when the pH is decreasing. As much as 12% of nitrogen was released as ammonia during four weeks of storage of a fermented silage of Baltic herring at 28°C (Nilsson and Rydin, 1963). Corresponding figures with acid-preserved silages were 3% (Gildberg and Raa, 1977). Nevertheless, the ammonia must derive from non-essential amino acids, since the nutritional value of fermented silage was very good (Kompiang, Darwanto and Arifudin, 1980). They reported that the nutritional quality of lactic acid fermented silage was significantly better than acid silage when fed to chickens. It is likely that this is due to protective action by the fermentation process on fish lipid.

However, problems of taint and off-flavour have been reported. Wirahadikusumah (1969) reported that broilers picked up an off-taste when fed 40 g wet fermented silage per bird per day, but this problem was circumvented by omitting silage from the ration in the last week before slaughter at eight weeks old. Olley and James (1972) found that a faishy taint developed in pigs fed fermented fish silage. They suggested that silage products must be used carefully.

<u>Conclusions</u>

Products prepared by biological fermentation have the advantage that they do not require the use of acids which are dangerous and expensive. However, considerable know-how is necessary and the must be tried out carefully for each type of raw material and under the particular local conditions. Larger scale trials should be carried out to test the technical and economic feasibility of the process. There is a considerable difference between the laboratory experiment described above and the operation of a commercially viable enterprise.

III - Dried Fish Silage

Conventional wet silage is appropriate in situations where the site of production is close to the site where the feed will be used. However, in many countries (especially in the Tropics) this is not the case and the storage, handling and transportation of wet silage would be both difficult and expensive, particurly where the raw material was only available at seasonal intervals. A further problem would be the high oil content of many small tropical species, which would complicate the use of a liquid product. Another complication is the need to make the fish suitable for use at the village level as well as being suitable for more commercial animal production systems. In either event a more complete animal feed would be produced if an energy source could be incorporated into that silage. Comparatively cheap vegetable products such as cassava, barley, maize, rice bran and groundnut husks are often available in many countries. Such cheap agricultural products could be used to prepare a cheap animal feed using simple equipment and requiring a low technological input.

Preparation

Silage/carbohydrate products can be prepared from waste fish using either formic acid or a cheaper combination of mineral acid and formic acid. The required pH level for preservation is dependent upon the species of fish used and climatic conditions. Under extremely poor drying conditions an initial pH of 2.0 with 1% formic acid added was found to be necessary but under more favourable drying conditions, a higher pH and less formic acid would be acceptable (Disney, Tatterson and Olley, 1977).

Disney and Hoffman (1976) suggested that the optimum combination of pH and formic acid must be determined by experiment, but pH 3.0 and 0.5% formic acid have been agreed to be generally suitable. After acidifying the fish mince, complete liquefaction will occur in two to four days. A dry source of energy such as cassava, rice bran, etc is then added and the resulting paste is dried and finally broken up by hand.

After that the product should be stored in watertight plastic bags

or tightly closed plastic containers. Under tropical conditions sun drying would be used, but the effectiveness of drying is dependent upon the prevailing weather conditions. Many countries have a prolonged wet season and drying could be delayed. Drying indoors away from direct sun with little air movement is an alternative in wet areas, but it needs a longer drying period. To achieve stability during storage a moisture content below 10% should be the aim. The product has been stored for one year without deterioration although fat oxidation will affect the quality of the product (Disney and Hoffman, 1976). The product should be stored in mosture-proof bags or containers to prevent the absorption of moisture, but is otherwise easily stored and transported.

Nutritional value

A variety of dried fish silage products have been reported (Krishnaswamy *et al*., 1965; Atkinson, *et al*., 1974; Disney, Tatterson and Olley, 1977; Disney and Hoffman, 1976; Rattagool *et al*., 1980; Poulter *et al*., 1980; Kompiang, Arifudin and Raa, 1980). The results of these experiments and feeding trials suggest that silage/carbohydrate products can be fed to animals (especially poultry) as a complete diet or mixed with other raw materials. However, problems of poor palatability and growth have been reported (Luscombe 1973; Disney *et al*., 1978). The poor results obtained with stale and spoiled fish may be due to the formation of histamaine and other irridazole compounts (Olley, 1972). Also no attempt was made in any experiment to remove the oil from the silage, therefore unsaturated fish oils could create problems of taint and palatability. In addition rancidity reactions may lead to the destruction of the fat-soluble vitamins in the ration. For example, rancid fats destroy tocopherols which can then no longer protect vitamin A in the gut (Jensen, 1973).

In spite of these problems, the evidence is that dried fish silage products could be used as a valuable source of protein and energy for compounding feeding rations for poultry. This could considerably reduce animal feed imports in many developing countries.

IV - Pathogens and Toxins in Fish Silage

Although no systematic research has been undertaken, it is likely that pathogenic bacilli and clostridia (that cause such diseases as cholera, typhoid and anthrax) are killed by the low pH of fish silage.

Neither has the stability of botulin toxin, which might be present in raw material prior to ensiling, been examined. Survival times of viruses and parasites in fish silage have yet to be determined (Raa and Gildberg, 1982).

Gaiger (1978) reported that silage made with 3% formic acid sometimes becomes infected by moulds, in particular *Aspergillus flavus*. Strøm *et al*. (1980) reported that it seems safe to heat the silage prior to use and recommended the use of a combination of formic and propionic acids to prepare a good silage. Bacterial growth is then inhibited as well as growth of moulds including *Aspergillus flavus*. This is of great importance when preparing silage in tropical regions. The presence of bacterial toxins in the silage is not a problem when preparing silage from fresh raw material. However, there is a risk of toxins, e.g. toxin from *Clostridium botulinum* being present when ensiling spoiled raw material. The possible thermal destruction of both toxins and spores from *C. botulinum* under the conditions used for lipid removal (95°C) is under investigation.

Salmonella bacteria are apt to persist in dry animal feed (Hauge, 1967). However, this does not appear to be the case with the chicken feed containing viscera silage (Salte and Hellemann (1982). Their results show that *Salmonella typhimurium* perish within two weeks in chicken feed containing viscera silage stored at 20°C.

In addition to microbial toxins, the presence of toxins from poisonous fish like puffers may be a problem in tropical waters when preparing silage from shrimp trawlers by-catch. There is no information regarding the stability of such toxins in fish silage.
The process by which the puffers become toxic has not been fully revealed, but it seems that the toxin derives from poisonous marine algae (Dinoflagellates), which may explain why the toxicity of

puffers varies with the locality and season. Irrespective of the degree of toxicity, however, puffers represent a constant hazard and should be removed before any processing of the remaining catch, unless it can be demonstrated that the toxin is degraded during processing. This is an important area for further studies. For more details about toxicity of puffers see review by Koa (1966).

V - Economic Aspects of Production

The main advantage of the fish silage process is that in areas where there is no established fish meal factory, fish offal and waste fish can be utilised instead of being thrown away.

The capital investment in a silage plant is considerably less than for a similar sized fish meal plant, although the internal rate of return is similar. Consequently the 'risk' element on investment in fish silage production is greatly reduced. The monetary return to a supplier of raw material, either boat owner or processor, is at least equal to that obtained from a fish meal producer, and at best could be increased.

The advantages and disadvantages associated with fish silage were discussed in a previous part of this review. Despite those disadvantages an investment in silage could be profitable in an area with no outlet for waste fish or offal given the right circumstances of continuity in raw material supplies and farm location.

A number of papers have been published on the production and feeding of fish silage, although only a few have considered the economic aspects (Disney, Tatterson and Olley, 1977; Nicholson, 1976, Gaiger, 1978; Disney, 1979 and Edwards and Disney, 1980).

Nicholson (1976) reported that the costs of industrialised silage production competes favourabley with fish meal, mainly as a result of lower capital investment. The price in 1976 of silage plant with oil separation equipment was about half of that of a fish meal factory with the same capacity. The production costs per tonne raw material of de-oiled silage preserved with 3% formic acid were also lower than for fish meal. This was mainly due to saving in labour costs, since the silage process does not require a 24 hour shift system. There was also considerable saving in fuel costs, but this was counteracted by the costs of formic acid. The main disadvantage of silage compared to fish meal is the cost of transportation from the plant to the consumer. However, it was shown that up to a radius of about 80 miles, production and distribution of wet silage was more economical per unit of protein than was fish meal. This distance can be subject to contradiction depending on the selling

price and protein content of the fish silage when marketed.

Disney _et al_. (1978) investigated the cost of producing a dried fish silage (fish silage/carbohydrate) in both Ghana and Malawi. In Ghana, using the whole price for fish and cassava, and including estimated costs for equipment, labour and transport, it was calculated that one ton of product containing 20% protein would cost between £35 to £45. By comparison, fish meal (60% protein) was being imported, at that time (1974), at £108 per tonne. In terms of protein, the silage/cassava product is hardly competitive but it already contains the energy component of an animal feed which must be added to fish meal and the cost of the raw material was quite high. Therefore the silage product would cost substantially less.

In Malawi, 10 kg of fish, together with 2 kg of ground maize, was found to yield 5.5 kg of product containing 30% protein. Including materials, labour, equipment and transport, it was calculated that 1 tonne of product would cost approximately £75 to produce. At that time (1976), fish meal containing 60% protein was being imported at £250 per tonne. Thus the silage/carbohydrate product would be highly competitive. The purchase of fish accounted for nearly two thirds of the production cost. They suggested that where waste fish is available at negligible cost, the silage carbohydrate product may be used for as little as £30 per tonne.

From the information collected in Ghana and Malawi it would appear that a commercially viable operation may be possible under certain circumstances.

In Mexico, Edwards and Disney (1980) investigated the production cost of silage from shrimp by-catch. Their results showed that silage protein was cheaper than alternative sources of protein (soya, pressed cake, fish meal) for use by the local pig industry.

In Sri Lanka, Jayawardena _et al_. (1978) investigated the cost of production of a dried fish silage/rice bran mixture for use as a poultry feed. Disney (1979) has extended the analysis by assessing its value on the basis of a least cost formulation for a poultry feed using material available in Sri Lanka.

The future of industrialised fish silage and fish meal production depends vitally on the prices of the preservative acids and of oil. It is a realistic estimate that the price of both will continue to rise in the future. The strict economical calculations are relevant primarily for the industrialised version of salvaging waste fish and resources which otherwise are discarded. For the village fishermen and farmers in many developing countries, however, such calculations are not so important as the fact that the fish silage process is the only method available for the preservation of surplus fish and fish waste.

SECTION 5

PROTECTION OF DIETARY PROTEIN BY FORMALDEHYDE

Introduction

The ruminant animal derives its intestinal protein supply from dietary protein which escapes ruminal degradation (UDP), microbial protein which is synthesised in the rumen and from endogenous protein (ARC, 1980). The combination of amino acids derived from these three sources constitutes the total supply to the animal. While microbial protein alone may be adequate for low producing ruminants it is inadequate for supporting higher levels of growth, wool or milk production. Medium to high-producing ruminants rely on some dietary protein escaping degradation in the rumen and as production levels increase, so must the supply of UDP.

Probably little can be done to influence directly the amino acids provided by the endogenous proteins, but amino acids supplied by microbial protein and dietary protein can be modulated (Chalupa, 1973). There are various methods which can be used to protect dietary proteins from ruminal degradation. These methods are as follows:

1. Heat treatment
2. Chemical treatment
3. Encapsulation
4. Use of amino acid analogues
5. Inhibition of *in vivo* proteolysis of proteins
6. Oesophageal groove closure.

These different methods of protein protection have been reported and discussed in many recent reviews (Chalupa, 1974; Beever and Thomson, 1977). This report will discuss only the chemical protection of dietary protein using formadehyde.

Chemistry of the reaction

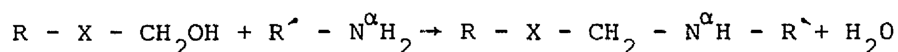
The general effect of HCHO on proteins has been reviewed by Barry (1976). In most cases the initial step appears to be the rapid formation of methynol compound.

$$R - XH + HCHO \rightleftharpoons R - X - CH_2OH$$

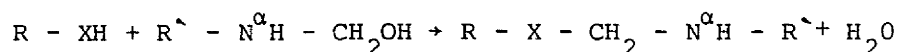
XH in the above formula can be any of the following:

a terminal amino group, the Σ-amino group of lysine, the primary amide groups of aspargine and glutamine, the guanid group of arginine, the hydroxy groups of threonine and serine, the sulphydryl group of cysteine, the phenyl group of phenyl alanine, the indole group of tryptophan, or the imadazole group of histidine.

Condensation reactions then occur slowly over a period of time, resulting in a formation of stable methylene cross-linkage between protein chains which decrease protein degradability at the pH of the rumen.



$$R - X - CH_2OH + R' - N^{\alpha}H_2 \rightarrow R - X - CH_2 - N^{\alpha}H - R' + H_2O$$



$$R - XH + R' - N^{\alpha}H - CH_2OH \rightarrow R - X - CH_2 - N^{\alpha}H - R' + H_2O$$

The methylene linkage are considered to be hydrolysed under the acid pepsin conditions in the abomasum. The liberated proteins are subsequently digested and the products are absorbed from the small intestine.

Effect on protein digestion

Formaldehyde (HCHO) is a well-known sterilising agent and is commercially available as formalin which contains about 40% of the gas in aqueous solution.

Watson (1939) reported that HCHO was used successfully as bacterio stat in silage making. Renewed interest in the use of HCHO as a silage preservative occurred some forty years later as a result of the studies of Brown and Valentine (1972). Many workers were interested in the additive because it reduces significantly the rate at which rumen micro-organisms can attack the grass protein. Hoever, the level of HCHO is critical because, mild protection may have little effect on the rate of breakdown of the protein in rumen, yet drastic treatment may result in denaturing the protein and reducing the digestibility in the small intestine.

(a) Protein sources of animal origin

Ferguson *et al*. (1967) compared the *in vitro* solubility at pH 6 of untreated casein with HCHO-treated casein. Treatment reduced the solubility from 83 to 8%. In an *in vitro* digestibility trial, 89% of the untreated casein was degraded by rumen organisms in 24 hours whilst only 4% of the treated casein was degraded in the same time. In an 18 week wool-growth trial, sheep receiving a supplement of HCHO-treated protein produced 70% more wool than the casein-fed control. Offer *et al*. (1971) investigated the effect of HCHO-treatment of casein on the flow of nitrogenous materials through the duodenum of sheep during a 24 day continuous collection experiment. Treatment of casein resulted in an increase in the flow of total amino nitrogen and most individual amino acids to the duodenum.

HCHO has been long used to preserve fish intended for the production of fish meal. However, investigations of the nutritive value of fish meal prepared from fish preserved with HCHO have been largely directed towards examining whether the nutritive value has been adversely affected and no attention has been paid to the question of protein protection by the preservative (Ferguson, 1975).

(b) Protein sources of plant origin

While treatment of plant protein, e.g. peanut protein, soya bean protein, has not yielded consistent responses (Amos *et al*., 1974; Schmidt *et al*., 1973) growth rates and feed conversion efficiencies have been improved in some trials (Faichney and Davies, 1972; Hatfield, 1973; Nimrick *et al*., 1972; Wikoff *et al*., 1972). Treating plant protein with HCHO usually increases faecal nitrogen excretion and decreases excretion of urinary nitrogen; the latter decrease probably being due to decreased absorption of nitrogen as the level of HCHO increased (Amos *et al*., 1974). The magnitude of the faecal increase appears to be less severe with casein than with plants protein. Perhaps the failure to obtain a consistent increase in nitrogen retention from protection may be due to the biological value of plant protein being lower than that of animal proteins such as casein.

Adding HCHO to forages at ensiling appears to promote consistent

increases in animal performance (Barry et al., 1972; Brown and Valentine, 1972; Waldo et al., 1973).

Wilkins et al. (1974) have examined in detail the effects of different levels of formalin on the composition and nutritive value of grass silages.

Responses by treating forages with HCHO at ensiling probably are due in part to decreased ruminal degradation of resultant silage protein, but the effects of HCHO upon silage fermentation also must be considered. Formaldehyde treatment may reduce proteolysis during silage fermentation and may also prevent excessive acid formation (because of its bactericidal properties). Consumption of dry matter of well preserved silages is negatively related to the degree of fermentation during ensiling (McDonald and Whittenbury, 1973) and adding inhibitory materials such as formic acid and HCHO does increase silage consumption (Brown and Valentine, 1972).

Summary

The results presented in Table 19 refer to several experiments in which dietary protein was protected by the use of HCHO and detailed measurements were made of protein or nitrogen flow into, and absorption from, the small intestine (cited in Beever and Thomson, 1977). The response to HCHO treatment compared with the untreated diet (in terms of the amount of protein flowing into the small intestine) varied between -3% from peanut meal to + 64% for casein when the responses were calculated on the basis of equalised intakes of protein.

In those experiments in which flow at the ileum was measured and the quantity of protein apparently absorbed from the small intestine was determined, wide variation in the response to HCHO was noted.

Conclusions

Protection of dietary protein from ruminal degradation enables more amino acids to reach the intestine than otherewise would be the case and, therefore, provides more adsorbable amino acids per unit of absorbable energy.

Formaldehyde treatments of proteins offers a potential method to

protect protein from ruminal degradation. However, more research is required on formaldehyde treatment procedures and the optimum level of treatment for specific proteins should be determined. It is vital that procedures do not interfere with other aspects of ruminal metabolisms or post-ruminal digestion and absorption. It seems that optimum conditions to treat plant protein with formaldehyde without affecting microbial metabolism, microbial protein production or intestinal digestibility and absorption may be difficult to achieve.

T A B L E 19

Effect of formaldehyde on the flow of protein into, and the absorption from, the small intestine, and the digestibility of protein within the small intestine

Diet component treated	Casein		Casein		Peanut meal		Peanut meal		Grass (ensilage)		Soya bean meal		Clover hay		Grass (dried)			
Diet component*(%)	40		30		33		50		100		55		100		100	100		100
Total diet HCHO Application rate (g/100g CP diet component)	1.2		3.0		0.9		0.9		6.0		0.6		4.0		1.0	2.0		3.0
Species	Sheep		Sheep		Sheep		Sheep		Sheep		Cattle		Sheep		Sheep		Sheep	Sheep
	U**	T**	U	T	U	T	U	T	U	T	U	T	U++	T++	U	T	T	T
g protein/24 h:																		
(a) consumed	231	234	170	170	102	110	187	194	161	153	-	-	179	180	136	142	138	133
(b) entering small intestine	136	226	135	159	132	138	154	197	133	169			126	187	182	229	187	178
(c) leaving small intestine	ND	ND	42	47	ND	ND	ND	ND	33	56	ND	ND	ND	ND	52	49	61	57
(d) absorbed	ND	ND	93	112	ND	ND	ND	ND	100	113	751+	897+	90+	144+	130	180	126	121
Digestibility (d/b)%	ND	ND	69	70	ND	ND	ND	ND	75	67	ND	ND	ND	ND	71	78	68	68
Reference	1		2		1		1		3		4		5			6	6	6

Footnotes:

* With respect to nitrogen in the diet and in the treated component
** U = Untreated; T = Treated
\+ Absorption from small and large intestine
\+\+ Nitrogen or non-ammonia nitrogen x 6.25
ND Not determined

* Beever and Thomson (1977)

Reference:

1. Faichney, 1974
2. MacRae *et al.*, 1972
3. Beever *et al.*, 1977
4. Verite and Journet, 1977
5. Hemsley, Hogan and Weston, 1970
6. Thomson and Beever, unpublished data

CHAPTER TWO

MATERIALS AND METHODS

DETERMINATION OF DRY MATTER

Dry matter estimates of feedstuffs were carried out by heating triplicate samples of 5 g in preweighed dry crucibles in an oven at 100°C for at least 24 hr. The crucibles were then transferred to a desiccator to cool then reweighed, and the dry matter content calculated. Care was taken to avoid delay in weighing the dried samples after removal from the desiccator, since rehydration was found to take place.

For fish silage samples, approximately 20 g of sample in triplicate, were oven dried at 105°C for 20 hr to constant weight.

DETERMINATION OF pH

The pH was measured on freshly strained rumen fluid or fish silage using Cranwell*[1] pH meter (U.K.). The meter was calibrated using standard buffer solution of pH 7.0 for the rumen fluid and pH 4.0 for fish silage at 37°C and 20°C respectively.

COLLECTION OF RUMEN FLUID

The apparatus described by Alexander and McGowan (1966) was used (See Figure 15). It consisted of a 100 cm^3 syringe attached by polythene tubing to a non-return valve system (V). This enters a 1 litre Thermos*[2] flask through a rubber bung. Tube A which enters the flask by the same route, is inserted into the rumen via the cannula. The end of Tube A is sealed, but several holes are cut near the end (see Figure 15) to filter the rumen fluid. The minimum vacuum to induce the liquor to flow into the flask (F) is created by operating the syringe. When the filter holes block up, Tube A is withdrawn slightly and shaken to clear the blockage. The flask was quarter filled from each sheep at sampling (usually 2 sheep were used). Immediately after sampling the pH of the liquor was taken and the flask transferred to the laboratory.

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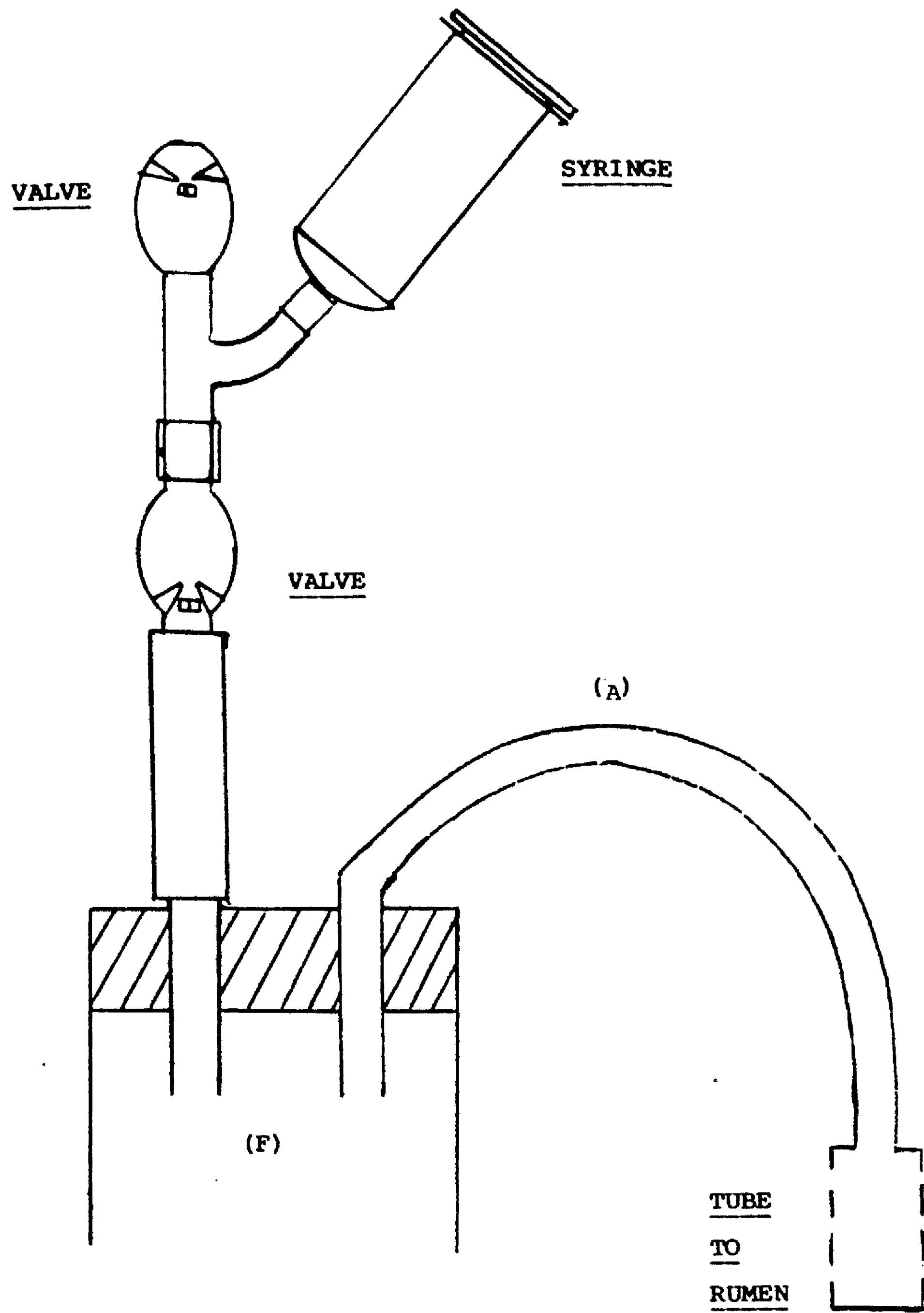
*[1] Cranwell, Brentwood, Essex, CM14 4XT, U.K.

*[2] Thermos Limited, England.

Figure 15: Collection apparatus for rumen fluid *



*ALEXANDER AND McGOWAN (1966)

DETERMINATION OF TOTAL NITROGEN IN FEEDSTUFFS

Total nitrogen was determined by the Kjeldhal method based on the technique described by Egan *et al*. (1981). Samples were digested with sulphuric acid using selenium oxide as a catalyst. Hydrogen peroxide was added to help to oxidise the organic matter in the feed. The second step was the Colorimetric estimation of ammonia released by adding alkali to the digest.

Reagents

1. Digestion mixture made up by mixing slowly with cooling the following:

   a. 40 g selenium oxide in 100 cm^3 distilled water.

   b. 2 litre concentrated H_2SO_4.

2. Buffer - 5 g NaOH + 3.74 g anhydrous Na_2HPO_4 + 31.8 g Na_3PO_4 12 H_2O + 10 cm^3 sodium hypochlorite (10-14% av.Cl) in 2 litre distilled water.

3. Caustic phenol - 2.4 g NaOH + 0.1 g sodium-nitroprusside + 20 g phenol in 1.6 litre distilled water.

4. Ammonia standards - ranging from 0.05 - 0.25 gl^{-1} ammonia nitrogen were made from a stock solution containing 4.7168 g $(NH_4)_2SO_4$ in 1 litre 10% H_2SO_4 .(1 g l^{-1} N). The different ammonia-N concentrations were made by appropriate dilution in 10% H_2SO_4.

Method

a. Digestion

About 0.1 to 0.15 g of test material was weighed precisely into a 75 cm^3 graduated digestion tube. 8.0 cm^3 of digestion mixture was added to each tube using an automatic dispenser, followed by three 1 cm^3 volumes of hydrogen peroxide (100 vol) and one piece of sintered glass. The tubes were transferred to a block digester*

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*Tecator Limited, Cooper Road, Thornbury, Bristol, BS12 2UP, U.K.

and heated progressively to 350°C for 2 hr. The tubes were then allowed to cool for 1 hr after which the contents were diluted carefully using 50 cm^3 distilled water and left to cool again. The volume was then made up to the 75 cm^3 mark with distilled water. The contents were mixed thoroughly and allowed to settle for 1 hr before samples were taken for analysis.

b. Ammonia analysis

Ammonia was measured using the Indo-phenol colorimetric method which proved both accurate and rapid in use.

Samples of constant volume (about 0.1 cm^3) either standards or digests, were dispensed into 50 cm^3 test tubes. 8.0 cm^3 of caustic phenol and 20.0 cm^3 of buffer solution were then added. Tubes were swirled and left to stand at room temperature for 1 hr for colour development.

Absorption at 585 nm was then measured using a spectrophotometer model, SP8-500 (PYE)*. The measurement for the blanks and standards were used to draw a graph of nitrogen concentration against absorbance from which the nitrogen content of unknown was calculated. For high nitrogen feedstuffs such as casein, soya bean meal, fish meal etc., it is necessary to dilute the digest further or use smaller initial samples (less than 0.25 g).

DETERMINATION OF TOTAL FREE AMINO ACIDS IN RUMEN FLUID SUPERNATANT

The determination of total amino acids in supernatant was carried out using a spectrophotometric method based on measurement at 230 nm of the ultraviolet absorption of their copper salts. Casein hydrolysate was used as the standard rather than alanine as in the original method described by Spies (1952). This was because casein hydrolysate is cheap, highly soluble and contains a mixture rather than a single amino acid.

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* PYE Unican Limited, York Street, Cambridge, CB1 2PX., U.K.

Reagents

1. Copper chloride (0.05 M) - 8.25 g of reagent grade cupric chloride dihydrate in 1 litre distilled water.

2. Sodium borate buffer, pH 9.1 to 9.2 - 40.3 g of reagent grade, anhydrous sodium tetraborate in 4 litres of distilled water; filtered through Whatman No. 4 filter paper.

   For use with unknowns, 6.0 g of reagent grade sodium chloride was dissolved in 100 cm^3 of borate solution. Sodium borate solution containing no sodium chloride was used with the casein-hydrolysate standards, because the ultraviolet-absorbing linkage of copper salt of casein hydrolysate amino acids is relatively unstable in the presence of chloride traces already present in the casein hydrolysate.

3. Standards - casein hydrolysate (30 - 150 mgl^{-1}) freshly made up in distilled water.

Method

a. Development of Colour

To 5 cm^3 of an aqueous solution of an appropriate concentration of amino acids in a 25 cm^3 centrifuge tube was added 5 cm^3 of the buffer solution. Blank solution was made by mixing 5 cm^3 of water with 5 cm^3 of buffer. 0.1 cm^3 of copper chloride solution was then added, and the tube was shaken and left standing for 10 min at room temperature. The suspension was centrifuged at 2000 x g for 5 min and the clear supernatant was carefully decanted into a clean 10 cm^3 glass tube. The necessity of avoiding traces of copper and amino acids in reagents, distilled water or glassware is obvious. Test solutions containing copper could not be analysed because the blank solution would cancel the absorbance of the copper salts of amino acids. Also the pH control in the formation of the copper salts is essential. However, significant change in the pH on the addition of the test solution to the buffer solution would be unlikely in the present method because of the high dilution of the test solutions used.

b. Spectrophotometric analysis

The absorbance of the clear solution was determined at 230 nm using the spectrophotometer*[1] Special care was required to prevent contamination by traces of insoluble foreign matter which might interfere with the measurement. Good conformity to Beer's law over the entire range of casein hydrolysate concentrations (30-150 mgl^{-1}) was observed. The absorbancies were converted to concentration by reading them off the casein hydrolysate standard curve.

DETERMINATION OF AMMONIA-N IN RUMEN FLUID SUPERNATANT

Supernatants were analysed for ammonia-N using the same colorimetric procedure (phenol-hypochlorite) described earlier in total nitrogen determination (see page 139).

SAMPLING PROCEDURE FOR FISH SILAGE

The fish silage bulk (up to 200 l in each drum) was stirred and mixed thoroughly using a wooden paddle. Small portions (200 cm^3) were then taken from different levels using a milk sampling ladle and placed in a food mixer*, and well homogenised for 5 min . After that the fish silage was transferred to plastic buckets, sealed and kept at -20°C for subsequent analysis.

DETERMINATION OF ASH CONTENT OF FISH SILAGE

Approximately 20 g of samples in triplicate were heated in a muffle furnace for 24 hr at 500°C. The samples were cooled in a desiccator for 1 hr then reweighed and the ash content calculated.

DETERMINATION OF INSOLUBLE NITROGEN CONTENT (TRUE PROTEIN) IN FISH SILAGE

Determinations were carried out in triplicate. To approximately

*[1] SP8-500 (PYE) model

*[2] Waring Commercial Limited, New Hartford, Connecticut, 06057, USA.

5 g of sample 10 cm^3 of 20% (w/v) trichloroacetic acid (TCA) was added and after homogenisation of the mixture for 5 min the sample was filtered through a 2 cm nitrogen-free Whatman No. 541 filter paper.

The nitrogen content in the residue was measured by the Kjeldahl method. The true protein was calculated as residue N x 6.25.

DETERMINATION OF NON-PROTEIN NITROGEN (NPN) OF FISH SILAGE

Non-protein nitrogen was calculated as follows:

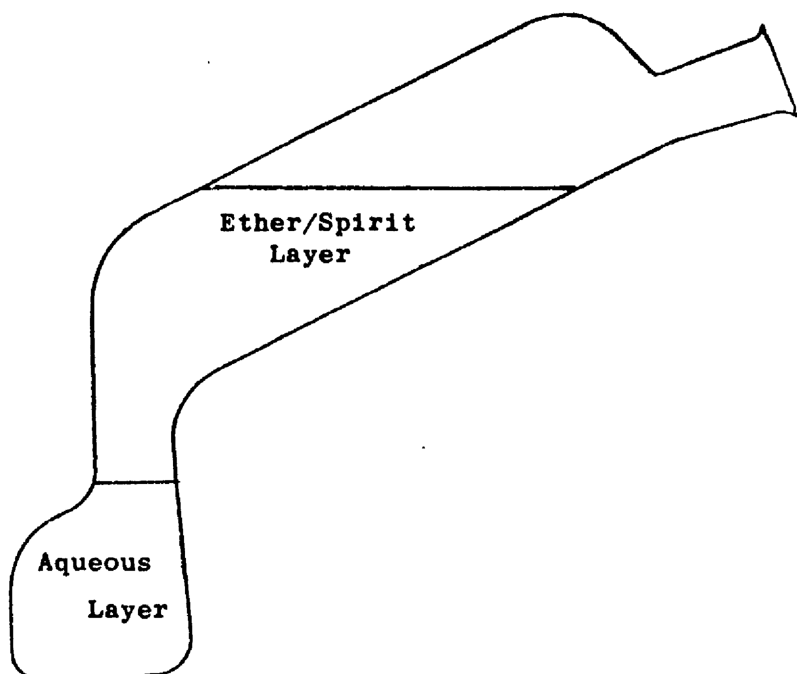
NPN = total nitrogen - insoluble nitrogen (TCA)

DETERMINATION OF OIL CONTENT OF FISH SILAGE

Oil content of fish silage was measured using the method of Majonnier (B.S. 1741, 1963). Approximately 10-11 g of fish silage was weighed into an extraction tube (see Figure 16), then 1 cm^3 of aqueous ammonia was added. The mixture was well homogenised for 1 min and 10 cm^3 of ethanol was added. The mixture was homogenised by allowing the liquid to flow backwards and forwards between the two bulbs. Complete extraction of the oil is dependent upon satisfactory mixing at each stage.

About 25 cm^3 of diethyl ether was added to the above solution and the extraction tube closed with a cork wetted with water before insertion, and the mixture shaken vigorously for 1 min. The cork was then removed and 25 cm^3 of light petroleum (petroleum spirit, b.pt 40°C - 60°C) added, using the first few cm^3 to rinse the cork and the neck of the tube so that the rinsings ran into the tube. The cork was replaced again and the mixture shaken vigorously for 30 sec . It is essential that the cork be wetted with water before each insertion and washed with solvent during each removal. Also, before each removal, to avoid spurting of the solvent a slightly reduced pressure should be induced in the tube by cooling it in running water.

Figure 16 Majonnier-type oil extraction apparatus



The tube was allowed to stand, usually for not less than 30 min ,
on the flat bottom of the lower bulb, until the supernatant layer of solvent was clear and completely separated from the aqueous layer. If the interface between the two layers was below the lower end of the narrow part of the tube, it was raised to this by adding carefully a little water down the side of the tube.

The cork was removed and the supernatant layer decanted carefully into a short-necked flask. The outside and the neck of the tube were rinsed with mixed solvent and rinsings collected in the flask.

The extraction of the oil from fish silage residue was repeated twice by using 15 cm^3 of diethylether and 15 cm^3 of light petroleum and the supernatants were transferred to the collection flask. The solvent was completely removed from the flask by distillation, then the flask heated in the oven for 1 hr at 100°C. The flask was allowed to cool in a desiccator for 1 hr and weighed. The heating was repeated for periods of 30 min until the weight no longer decreased.

The oil in the flask was extracted with successive 15 cm^3 quantities of light petroleum. After each addition the flask was warmed and the solvent swirled round the sides until all the oil appeared to be in the solution. After that, the solution was decanted carefully without disturbing any sediment. The flask was allowed to cool and then weighed, and the difference in weight before and after the extraction with light petroleum was the apparent weight of oil in the weight of fish silage taken.

Blanks were determined with 10 cm^3 of water and the apparent weight of oil was corrected by subtracting the bank value.

DETERMINATION OF THE FATTY ACID PROFILE OF FISH SILAGE OIL

During the study, the fatty acid profile of fish silage was determined according to B.S. 684 (1980). The method is based on transesterification of the glycerides by reaction with methanolic potassium hydroxide solution followed by gas liquid-chromatography (GLC).

Reagents

1. Potassium hydroxide - methanolic solution, approximately 2M was prepared by dissolving 11.2 g of KOH in 100 cm^3 of methanol containing not more than 0.5% of water.

2. Heptane - of chromatographic quality.

Equipment

1. Test tubes (5 cm^3), with ground glass stoppers.

2. Centrifuge test tubes (50 cm^3).

3. Graduated pipette.

4. PYE* 104 chromatograph with F.I.D. detector and integrator.

5. Hamilton micro-syringe.

Method

a. Preparation of oil sample

About 20 g of fish silage was placed into 50 cm^3 test tube and centrifuged at 2500 x g for 20 min . The upper layer containing the oil was removed carefully, well homogenised and kept at -20°C for subsequent analysis.

b. Preparation of the methyl esters

Approximately 20 mg of the separated oil was placed into the 5 cm^3 test tube, then 2 cm. of heptane was added. The mixture was shaken until the oil completely dissolved (20 sec). About 0.2 cm^3 of methanolic KOH was added and the test tube was stoppered. The mixture was well shaken until the solution became clear (30 sec). After that the solution became cloudy again as a result of the separation of glycerol which settled rapidly. The upper layer containing the methyl esters was decanted carefully into small containers and analysed by GLC as soon as possible.

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*PYE Unican Limited, York Street, Cambridge, CB1 2PX, U.K.

c. Gas liquid chromatography (GLC)

A 1 μl aliquot of sample was withdrawn from the methyl ester solution using the Hamilton micro-syringe. The sample was injected into the column of PYE 104 gas chromatograph equipped with a thermal conductivity detector. A glass column 183 cm^3 x 2 mm i.d. packed with Chromasorb (100-120 mesh) was used. The column temperature was 200°C (isothermal), and the injector, detector was not heated. The carrier gases were, N_2 (20 $cm^3 min^{-1}$), H_2 (20 cm^3 min^{-1}) and air (200 cm^3 min^{-1}). An electronic integrator was used to quantify the gas chromatographic results.

DETERMINATION OF ENERGY VALUE

Gross energy values were determined by combustion in oxygen in a ballistic bomb calorimeter (Gallenkamp, CG-370)*.

About 0.7 g of dried, milled and homogeneous sample was pelleted in a hand-pelleting machine and accurately weighed in a crucible of known weight. A platinum firing wire was connected to the bomb and a 50 mm white cotton thread was tied to the wire. The crucible was positioned inside the bomb on the support pillar and the other end of the cotton thread was rested on the sample. The bomb was then assembled, and slowly charged with oxygen to a pressure of 25 atmospheres. The galvanometer was brought to zero and the bomb was fired. The maximum deflection of the galvanometer was recorded and the gasses were released. All crucibles were examined for unburnt sample or carbon, then the bomb was dismantled and cooled.

The gross energy of samples was read from standard curve derived from the ignition of known weights of thermochemical grade benzoic acid also using 50 mm cotton threads. It proved necessary to recalibrate the calorimeter on each day that it was used.

PREPARATION OF CHROMIUM-MORDANTED FISH MEAL

The method of Elimam and Ørskov (1984) was used for mordanting fish meal particles with Cr as follows:-

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*Gallenkamp, 41 Gatwick Road, West Sussex, RH10 2RE, U.K.

Sufficient sodium dichromate ($Na_2Cr_2O_7$) to give 4% chromic oxide in the crude protein dry matter was made into a solution with water and added to the fish meal. It was then thoroughly mixed, using a Hobart*[1] food mixer with additional water added as necessary to give a firm paste. The resultant paste was placed in a shallow baking tray to a depth of 20 mm and covered with 2 mm of water, then sealed with aluminium foil and baked for 24 hr at 100°C. The dried material was then thoroughly washed with cold running water to remove excess chromic oxide, characterised by a green colour. When the rinsing water was clear, the material was re-suspended in water and left to stand for 1 hr. Ascorbic acid was added to lower the pH from 9.0 to 4.0.

The suspension was then left for 24 hr with occasional stirring and additional ascorbic acid was added as necessary to maintain the pH at 4.0 and to bring any loosely attached chromic oxide into solution. The suspension was then rinsed again and dried at 65°C. The resultant fish meal was intended to have a rumen degradability and water solubility of zero (Elimam and Ørskov, 1982 a, b).

Shortly before dosing the animals, 50 g of Cr-treated fish meal was transferred to a 50 cm^3 beaker and suspended in warm water. Sufficient carboxymethyl cellulose and sugar-syrup*[2] were then added to give a viscous liquid and to mask the taste of the Cr-treated fish meal. The mixture was well homogenised using a food mixer and used immediately.

PREPARATION OF CHROMIUM-EDTA COMPLEX

The Cr-EDTA complex was prepared using the procedure of Binnerts *et al*. (1968).

About 14.2g of chromic trichloride ($CrCl_3 . 6H_2O$) was dissolved in 200 cm^3 distilled water. A solution of 200 g disodium salt of ethylene-diamino tetra acetic acid in 300 cm^3 distilled water was

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*[1] Hobart Manufacturing Company Limited, Hobart Corner, New South Gate, London, N11 1QW, U.K.

*[2] Golden Syrup, Safeway Limited.

added to the first solution. The mixture was heated until boiling with a few carborundum chips for about 1 hr. The solution developed a deep violet colour as the 1:1 complex of EDTA and Cr was formed. After cooling, the small excess of EDTA was neutralised with calcium chloride* to pH 6-7, and the volume was made up to 1 litre exactly. The solution was stored in a polyethylene container until needed. For the calf, a volume of 250 cm^3 of the solution (about 1 g Cr) was suitable for use in passage-rate measurement.

DETERMINATION OF CHROMIUM SESQUIOXIDE IN FAECES

The determination of chromic oxide in the faeces was carried out using the modified wet digestion method of Stevenson and Clare (1963).

Reagents

1. Acid mixture - made up by mixing slowly, with cooling, the following:
   a. 250 cm^3 conc H_2SO_4
   b. 250 cm^3 conc H_3PO_4 (SG 1.75)
   c. 500 cm^3 distilled water
   d. 50 cm^3 10% $MnSO_4$ solution
2. 4.5% Potassium bromate
3. 0.45% Potassium bromate
4. Colour reagent - made by filtering an 0.25% solution of 1.5 diphenyl-carbazide in ethanol into 10 volumes of 0.25 M H_2SO_4
5. Potassium dichromate standards (1.936 gl^{-1} 1 mg cm^{-3} Cr_2O_3) diluted to give a range of 0.50 - 5.0 $\mu g\ cm^{-3}$ Cr_2O_3.

Method

The method involves two distinct processes:

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*Excess of Ca-EDTA and Ca do not interfere with the principle of determination; moreover, the chromium complex is very stable and does not decompose with calcium.

a. The quantitative oxidation of Cr_2O_3 to dichromate ion $Cr_2O_7^{-2}$.

b. Estimation of the dichromate ion concentration.

A known weight of sample, containing approximately 1 mg of chromic oxide, was heated at 550°C for 24 hr. The resulting ash was quantitatively brushed into a dry 50 cm^3 conical flask and the crucible was washed with two 3 cm^3 portions of the acid mixture and the washing was added to the conical flask. The flask was heated on a hot plate until the mixture boiled. After that 3 cm^3 of 4.5% $KBrO_3$ solution was added and boiling continued until production of bromine ceased. The flask was then allowed to cool at room temperature for approximately 10 min . A dilute solution of potassium bromate was then added (20 cm^3 of a 0.45% solution) and the mixture was boiled for a further 3-4 min to complete the oxidation. After cooling to room temperature, the solution was made up to 100 cm^3 with distilled water and allowed to stand overnight. By morning, insoluble material had sedimented, leaving a clear golden yellow solution. A sample of about 2 cm^3 of this supernatant was taken by pipette into a 50 cm^3 test tube, then 5 cm^3 of the colour reagent was added. All tubes were swirled to mix the sample and the standard with the reagent for colour development.

Absorption at 550 nm was then measured using spectrophotometer model SP8-500 (PYE). The measurements for the blanks and standards were used to draw a graph of Cr concentration against absorbance. The Cr concentration of the samples can then be read off the graph.

CHAPTER THREE

PREPARATION AND *IN VITRO* EVALUATION OF FISH SILAGE

SECTION 1

CHEMICAL COMPOSITION OF FISH SILAGE MADE FROM WHITING OFFALS AND THE EFFECT OF FORMALDEHYDE TREATMENT ON PROTEIN AUTOLYSIS

OBJECTIVE

The aims of this experiment were to study the chemical composition of fish silage made from Whiting offals and to determine if treatment with formaldehyde affects enzymatic breakdown of fish tissue protein.

EXPERIMENTAL

Raw Materials

The raw material for fish silage consisted of fish offals and fish scrap from white fish. It was of the type commonly marketed as feed for fur animals, consisting mainly of the clavicula, backbone, ribs, tail, adherent flesh, heads and viscera with roe and livers. The raw materials were collected from a plant which processes fish for human consumption. Immediately after collection, the raw materials were divided into two parts. One was used for the first batch and the other was packed in plastic bags and immediately frozen at -20°C for the second batch.

Preparation of Fish Silage

First batch

The fish silage was prepared in the laboratory from the fresh white fish filleting waste. The waste was chopped through a grinder with disc holes of about 10 mm, and the fresh mince was then mixed immediately with 2% (v/w) of 90% formic acid. The acid was thoroughly dispersed throughout the minced fish to avoid untreated material where spoilage bacteria could grow.

The acidified mince was divided into six parts each of 1 kg, and

T A B L E   2 0

Levels of formic acid and formalin used to treat fish waste

Treatment No.		Formic acid added (cm^3 kg^{-1} fish waste)	cm^3 of formalin kg^{-1} fish mince
1	Batch 1	20	0 (control)
2		20	1
3		20	2
4		20	3
5		20	4
6		20	5
7	Batch 2	15	0 (control)
8		15	10
9		15	20
10		15	30
11		15	40

different levels of formalin (40% formaldehyde) were added. The formalin levels used are shown in Table 20. The formalin was well mixed with the acidified fish mince and the six types of silage prepared were stored in 5 litre plastic containers covered with tightly fitting lids to prevent any loss of formaldehyde.

The six silages were stored in the laboratory at ambient temperature, viz 23°C (± 3°C) for ten days before any samples were withdrawn for chemical analysis and degradability measurements. The silages were stirred daily until liquefaction had occurred (about 2-3 days), but thereafter only when samples were taken.

Second batch

In the second batch fish silage was prepared using the same procedure described for the first batch but lower levels of formic acid were used (1.5% v/w). The effect of formaldehyde was again tested, but higher levels of formalin were used (see Table 20). The five silages were stored under the same conditions described in batch 1.

RESULTS

In the second batch the fish waste was treated with a range of high levels of formalin solution (10, 20, 30 and 40 cm^3 formalin kg^{-1} fish waste). All of the levels used were much stronger than would be advised for practical use. This resulted in a hard rubber-like product unsuitable for animal feeding because its physical form would not allow satisfactory mixing and distribution with dry diets. The results are therefore presented only for the silages made with the lower levels of formalin.

In the first batch where lower levels of formalin were used (1, 2, 3, 4 and 5 cm^3 kg^{-1} fish waste) a liquid product was obtained with all levels of formalin. This product has been proved acceptable to sheep when mixed with a dry complete diet based on straw and barley.

Analysis of all six types of fish silage (treatment 1, 2, 3, 4, 5 and 6) was carried out at the end of the storage period (ten days).

Dry matter content

The results are shown in Table 21. The dry matter content of the different types of fish silage is similar to that of raw material from which they were made, apart from slight dilution caused by the addition of formic acid and formalin. The six silages contain an average dry matter of 239 g kg^{-1} fresh weight. The results from the analysis of variance indicated that these were significant differences ($P < 0.05$) between treatments, probably due to sampling errors of the heterogenous fish silage. Analysis of variance can be found in Appendix 2.

Crude protein

The crude protein content in the dry matter of the six fish silages are within the range 541 to 574 g kg^{-1} DM. They contain an average of 55.6 g kg^{-1} DM. There were no significant differences between treatments.

Ash content

The ash contents shown in Table 21 fall within the range 138 to 157 g kg^{-1} DM with an average of 147 g kg^{-1} DM. Results of the analysis of variance showed no significant differences between any of the treatments.

Oil content

The oil content of silage was measured for treatments 1 and 6 only because they were chosen as protein supplements for a subsequent feeding trial (see Chapter 4, Section 1). The oil content was high due to the inclusion of viscera and liver in the raw material (white fish waste) which contains a high level of oil. Both treatments contained an average of 202 g kg^{-1} DM of oil.

Ammonia-nitrogen content

The levels of ammonia-N as a percentage of the total crude protein were very low in all fish silages (see Table 21). This indicates that the silages were free from the action of spoilage bacteria and fairly stable. However, it was noted that the amount of ammonia-N increased slightly after one month of storage, probably due to deamination reactions catalysed by endogenous enzymes in the raw

material. Levels of ammonia-N decreased with the increases of formalin level (see Table 21).

pH

The pH levels were measured over the storage period (ten days). The pH levels remained low at about 3.80 for all treatments.

True protein and non-protein nitrogen

The amounts of true protein were measured by precipitation with trichloroacetic acid (TCA) and the results are shown in Table 21.

In the case of the untreated silage (treatment 1), at ten days a value of 87% TCA soluble nitrogen was reached. Incomplete protein solubilisation was obtained with all formaldehyde treatments which resulted in a marked significant increase ($P < 0.01$) in the true protein proportion.

True protein ($g\ kg^{-1}$ cp) was linearly related ($r = 0.99$) to formalin level ($cm^3\ kg^{-1}$ waste). Maximum TCA - precipitable protein was obtained with 5 cm^3 formalin kg^{-1} fish waste (treatment 6) (see Figure 17). Analysis of variance can be found in Appendix 1..

DISCUSSION

Fish silage composition

The content of dry matter, crude protein, oil and ash in fish silage would be expected to depend on type of raw material, processing method as well as on seasonal variations. The proximate composition of the fish silage produced is shown in Table 21. There were small but significant ($P < 0.05$) variations in dry matter content between the six treatments. However, this can probably be attributed to the lack of uniformity of fish silage samples used, rather than real differences in the raw material. The protein, oil and ash contents on a dry matter basis were not significantly ($P < 0.05$) different because they were produced from the same raw material.

The low levels of pH and ammonia-N indicated that all six treatments were stable and well preserved.

TABLE 21

Composition of different fish silages made from whiting offal*

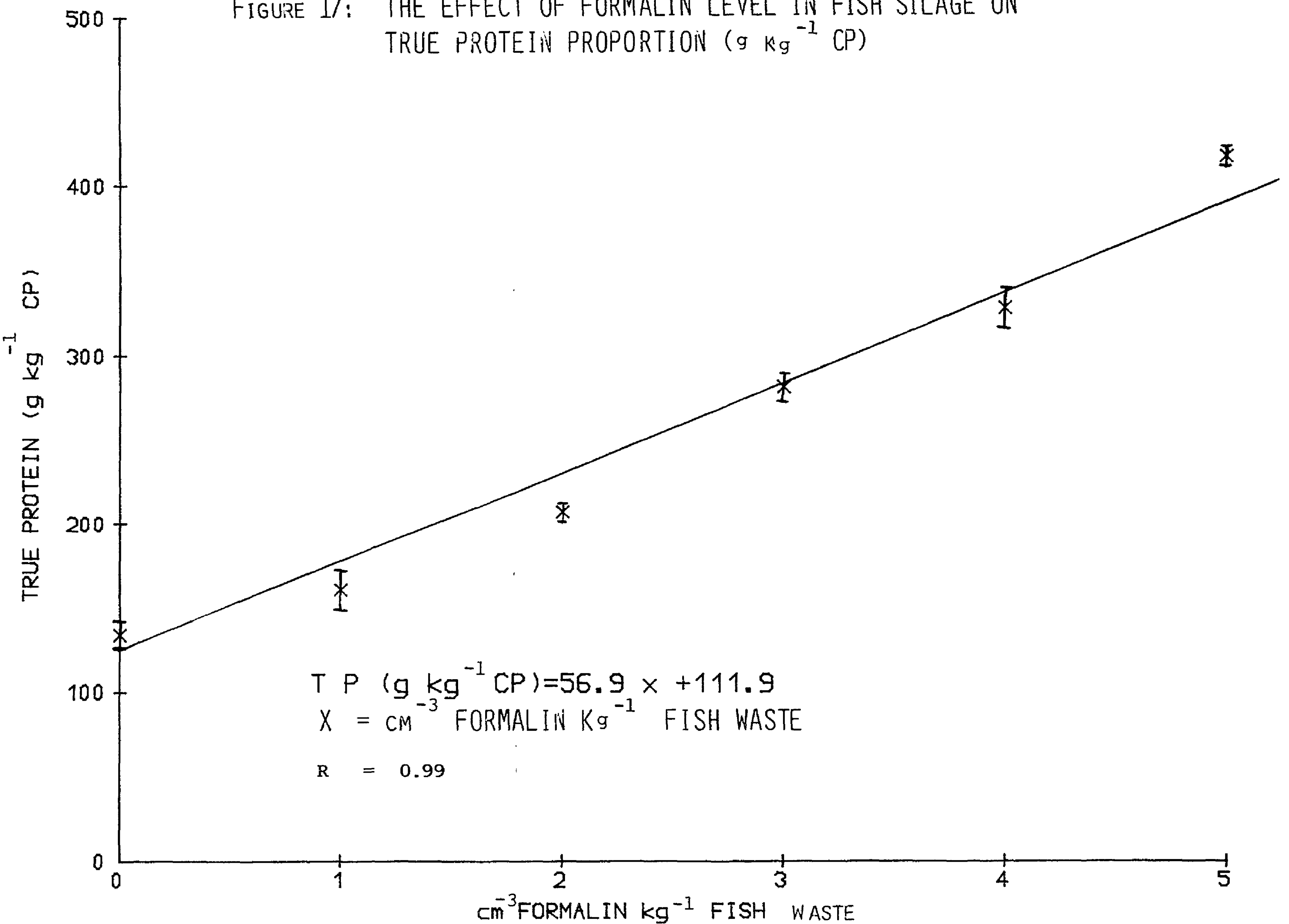
(measured after 10 days at 23°C

Teatement No.	cm^3 formalin kg^{-1} fresh waste	DM g kg^{-1} fresh weight	Ash g kg^{-1} DM	Oil g kg^{-1} DM	CP g kg^{-1} DM	NH_3-N g kg^{-1} CP	True protein g kg^{-1} CP	Non-protein nitrogen(NPN)[+] g kg^{-1} CP
1	0	239^{ac}	138^{a}	197^{a}	546^{a}	1.08	133^{a}	867
2	1	236^{b}	140^{a}	ND	568^{a}	0.79	160^{b}	840
3	2	237^{ab}	145^{a}	ND	574^{a}	0.79	207^{c}	793
4	3	237^{ab}	146^{a}	ND	541^{a}	0.73	281^{d}	719
5	4	240^{c}	157^{a}	ND	564^{a}	0.72	327^{e}	673
6	5	246^{d}	153^{a}	206^{a}	545^{a}	0.58	416^{f}	584
Mean	-	239	147	202	556	0.78	-	-
SED ±	-	1.277	7.370	6.363	11.80	-	10.90	-

*Values not sharing common subscripts differs significantly ($P < 0.05$)

+NPN content obtained by difference

FIGURE 17: THE EFFECT OF FORMALIN LEVEL IN FISH SILAGE ON TRUE PROTEIN PROPORTION ($g\ kg^{-1}$ CP)



The composition of fish silage produced in this study (means of six treatments) was compared to the level of the major constituents in white fish meal (Table 22). The values of white fish meal are those given in ADAS Advisory Paper No. 7 (1973). The protein level in the white fish meal was higher than in white fish silage, because of the high oil level in the fish silage.

The total ash level in white fish meal is also higher than in white fish silage.

In Table 23 the mean composition of white fish silages prepared in this experiment was also compared with the typical composition of white fish silage reported by Tatterson (1976). The protein level was again lower in the silage produced in this experiment, due to the inclusion of offals which contain a high proportion of oil especially for the liver. The offal and liver were discarded from the raw material in the previously reported fish silage (Tatterson, 1976). The dry matter content and total ash level were similar.

The effectiveness of formaldehyde treatment of fish silage

During the storage of fish silage the protein is broken down by the proteolytic enzymes present naturally in fish tissue (mainly the gut), and the nitrogen in the silage becomes more soluble. The results of this study showed that with the untreated fish silage (treatment 1), the percentage of soluble nitrogen (NPN) rises very sharply to about 87% after ten days. After that there was negligible increase in the proportion of soluble nitrogen.

In the case of low formalin treatments (Batch 1) the application of formaldehyde produced a marked decrease in nitrogen solubility. The greatest effect was obtained with Treatment 6 (5 cm^3 formalin kg^{-1} fish waste) where the NPN proportion (as a percentage of CP) was lowered from 87% (Treatment 1) to 58% by the treatment with formaldehyde. The reason for the lower values obtained with formaldehyde treated fish silages was the inhibition of the proteolytic enzymes in the fish tissue caused by formaldehyde application. It was interesting to note that the low levels of formalin treatment did not greatly affect the physical form of the silage and all fish silages were fairly liquid and suitable for handling as animal feed.

TABLE 22

Proximate composition of white fish silage and white fish meal

Composition	White fish silage (made from offals)	White fish meal (ADAS, 1973)
Dry matter (g kg^{-1} fresh weight)	239	900
Crude protein (g kg^{-1} DM)	555	701
Oil (g kg^{-1} DM)	202	40
Total ash (g kg^{-1} DM)	147	241

TABLE 23

Proximate composition of two types of white fish silage

Composition	White fish silage (prepared in this study	White fish silage (Tatterson, 1976)
Dry matter (g kg^{-1} fresh weight)	239	211
Crude protein (g kg^{-1} DM)	555	710
Oil (g kg^{-1} DM)	202	24
Total ash (g kg^{-1} DM)	147	190

CONCLUSIONS

Formalin treatment of fish silage increased the potential nutritive value of the product as a ruminant feedstuff by reducing protein breakdown and increasing the proportion of residual true protein.

Optimum formalin application rate for fish silage was considered to be 5 cm^3 formalin kg^{-1} fish waste. This level partly inhibited the proteolytic enzymes in fish tissue and may also protect fish silage protein from rumen degradation.

SECTION 2

MEASUREMENT OF FISH SILAGE DEGRADABILITY USING THE DACRON BAGS TECHNIQUE

OBJECTIVE

The purpose of this trial was to investigate the degradation of fish silage protein by rumen micro-organisms, and the effect of varying formaldehyde treatments on degradation of fish silage protein.

EXPERIMENTAL

Animals

Three mature Suffolk cross weather sheep, approximate live weight 75 kg, and fitted with permanent rumen cannulae were used to estimate the degradability *in sacco* of untreated and formalin-treated fish silages. The animals were kept indoors in loose pens.

Diet

During the experimental period the animals were given a maintenance ration of 800 gd^{-1} hay and 200 gd^{-1} Ewbol sheep pencils* in two meals offered at 9.00 a.m. and 5.00 p.m. respectively. The compositions of the roughage and concentrate portions of the diet are shown in Table 24. Fresh water was given *ad libitum*.

Treatments

Eleven different types of fish silage were subjected to evaluation. The treatment design is shown in Table 20. Each treatment was replicated in the three sheep with the order being completely randomised. All fish silages were well homogenised before sampling.

Preparation of bags

Nylon (Dacron) bags, made of nylon filter cloth HSO 13 were supplied by Henry Simons, Box 31, Stockport, Cheshire. The bags were 200 mm x 150 mm in size with approximately 47 μ pore diameter. The seams were double-stitched to prevent any leakage of samples and

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*BOCM, UK.

TABLE 24

Mean composition of the diet fed to the sheep during the degradability trials

A - Composition of hay

Number of samples		3
		Mean
Dry matter	(g kg^{-1})	849
Organic matter	(g kg^{-1} DM)	929
Crude protein	(g kg^{-1} DM)	101
RDP	(g kg^{-1} DN)	61
UDP	(g kg^{-1} DM)	40
M/D	(MJ kg^{-1} DM)	7.9
D-value	(%)	52.4

B - Composition of concentrate (302 Ewbal pencils)

Protein (%)	Fibre (%)	Oil (%)	Ash (%)	Vitamin IU kg^{-1}			Minerals	
				A	D_3	E	Selenium mg kg^{-1}	Magnesium g kg^{-1}
14.0	13.5	3.0	9.8	5.000	2.000	7.5	0.2	12.0

the bottom of the bags were curved to prevent the samples collecting in the corners. The bags were clearly labelled in numerical sequence and were weighed to three decimal places.

Preparation of feed samples

Sufficient amount of untreated and formalin-treated fish silages were homogenised using a food mixer to provide representative samples. Approximately 15 g of fish silage was weighed into each bag using a plastic syringe. The bags were tightly tied with nylon fishing line to prevent spillage of the contents. The bags were incubated in groups of three, because the sheep could comfortably accommodate only three nylon bags at one time. The three bags were attached to a rubber bung by three strings which passed through a flexible plastic tube. The bungs were of specific size to fit the cannulae of the three sheep. At the same time as samples were being weighed into bags, three samples of approximately 20 g of each type of fish silage were weighed into pre-weighed crucibles and oven-dried at 105°C for 20 hrs. They were reweighed after cooling in a desiccator for determination of dry matter content.

Incubation of bags in the rumen

The three replicated batches were incubated at the same time, one in the rumen of each sheep for each period and then withdrawn. The bags were incubated immediately prior to feeding since it was easier to introduce them to the rumen at that stage.

Six incubation times were used viz, 0, 3, 6, 8, 16 and 21 hr with treatment 1 to 6 and 0, 3, 5, 8, 16 and 24 hrs with treatments 7 to 11.

After removal from the rumen all the bags were washed in cold running tap-water and were further cold-washed in a preset automatic washing machine. Zero time bags received the same treatment except they were not incubated in the rumen.

After washing, all bags were oven-dried at 65°C for 48 hrs. The bags were allowed to cool in a desiccator and weighed again to three decimal places. The incubated material was emptied into small plastic containers and stored in a desiccator for subsequent analysis.

Nitrogen determination of fish silage and residues after incubation

The total-N content in the different types of silages and the dried residual was determined in triplicates by the Kjeldahl method (see page 138). The ammonia-N content was determined colourimetrically using the phenate-hypochlorate method (see page 139). Approximately 0.6 g wet fish silage or 0.2 g dry residues were used for total-N determination. Measurements were made in triplicate.

From these measurements the dry matter and nitrogen losses from the bags was determined (see Tables 25 and 26).

RESULTS

Dry matter and nitrogen disappearance from bags

The percentage disappearance of dry matter from the nylon bags is shown in Table 25. Table 26 shows the simultaneous (%) disappearance of nitrogen from the bags following incubation in the rumen. The nitrogen content of each bag before incubation was calculated by multiplying the dry matter content of the bag by the nitrogen content of the dry matter determined by the Kjeldahl method. The nitrogen content of the material in each bag post-incubation was determined in the same manner. The nitrogen disappearance from each bag could then be calculated.

All levels of formalin addition significantly ($P < 0.01$) reduced the (%) disappearance of nitrogen and dry matter from the bags compared with the untreated fish silage (see Tables 27 and 28). At lower levels of formalin inclusion, there were no significant differences in nitrogen disappearance from nylon bags between treatments 2, 3 and 4. However, significantly ($P < 0.05$) lower nitrogen and dry matter disappearances were observed for treatments 5 and 6 than those obtained for treatments 2, 3 and 4. Nitrogen and dry matter disappearances were significantly ($P < 0.05$) higher with treatment 5 than treatment 6 (see Table 27).

There were no significant ($P < 0.05$) increases in nitrogen and dry matter disappearance associated with increasing time of incubation up to 21 hrs.

TABLE 25

Means of (%) dry matter disappearance following rumen incubation of fish silage in Dacron bags

Low levels of formalin

Treatment No.	Formalin levels (cm^3 kg^{-1} fish waste)	Number of sheep used	Incubation time (hr)					
			0	3	6	8	16	21
1	0	*	97.22	100.00	100.00	100.00	100.00	100.00
2	1	3	94.93	96.50	92.66	96.39	95.07	96.86
3	2	3	98.62	97.32	93.71	93.29	94.32	95.08
4	3	3	99.74	94.77	96.44	97.23	97.57	95.62
5	4	3	97.19	96.31	91.56	95.93	92.30	92.09
6	5	3	86.84	85.26	87.52	89.03	90.03	91.75

High level of formalin

Treatment No.	Formalin levels (cm^3 kg^{-1} fish waste)	Number of sheep used	Incubation time (hr)					
			0	3	5	8	16	24
7	0	*	97.03	100.00	100.00	100.00	100.00	100.00
8	10	3	66.55	59.77	64.52	64.10	67.20	64.21
9	20	3	57.04	55.14	59.72	56.57	54.65	53.73
10	30	3	56.39	54.38	54.60	53.79	53.07	53.96
11	40	3	53.29	56.26	51.70	50.16	53.90	50.57

*Disappearance of dry matter following washing only was so great that untreated fish silages were not incubated in the sheep. Losses were assumed to be 100%.

TABLE 26

Means of (%) nitrogen disappearance following rumen incubation of fish silage in Dacron bags

Low levels of formalin

Treatment No.	Formalin levels (cm^3 kg^{-1} fish waste)	Number of sheep used	Incubation time (hr)					
			0	3	6	8	16	21
1	0	*	98.65	100.00	100.00	100.00	100.00	100.00
2	1	3	98.00	98.98	97.95	99.16	98.96	99.25
3	2	3	99.35	99.04	97.91	97.75	99.20	97.82
4	3	3	99.88	97.57	98.54	98.73	98.98	98.01
5	4	3	98.08	98.18	95.46	97.92	96.43	96.88
6	5	3	88.67	91.07	92.63	93.24	94.48	96.07

High levels of formalin

Treatment No.	Formalin levels (cm^3 kg^{-1} fish waste)	Number of sheep used	Incubation time (hr)					
			0	3	5	8	16	24
7	0	*	98.04	100.00	100.00	100.00	100.00	100.00
8	10	3	56.80	57.55	61.41	59.16	65.01	61.86
9	20	3	23.24	28.75	41.40	36.17	40.84	39.54
10	30	3	33.84	43.10	41.61	38.79	43.37	42.34
11	40	3	35.05	47.27	41.74	42.47	43.82	45.60

*Disappearance of nitrogen following washing only was so great that untreated fish silages were not incubated in the sheep. Losses were assumed to be 100%

T A B L E   2 7

The effect of low level of formalin on the (%) disappearance of nitrogen and dry matter of fish silage in the rumen*

Treatment No.	Formalin level (cm^3 kg^{-1} fish waste)	Mean (%) nitrogen disappearance	Mean (%) dry matter disappearance
1	0	99.78^{a}	99.53^{a}
2	1	98.72^{b}	95.41^{bc}
3	2	98.51^{b}	95.40^{bc}
4	3	98.63^{b}	96.91^{b}
5	4	97.24^{c}	94.17^{c}
6	5	92.69^{d}	88.55^{d}
SED ±	-	0.489	0.914

*Means not sharing a common subscripts differ significantly ($P < 0.05$)

TABLE 28

The effect of high level of formalin inclusion on the (%) disappearance of nitrogen and dry matter of fish silage in the rumen*

Treatment No.	Formalin level (cm^3 kg^{-1} fish waste)	Mean (%) nitrogen disappearance	Mean (%) dry matter disappearance
7	0	99.77[a]	99.66[a]
8	10	60.31[b]	64.41[b]
9	20	34.99[c]	56.48[c]
10	30	40.61[d]	54.37[cd]
11	40	43.35[d]	52.64[d]
SED ±	-	1.918	1.143

*Means not sharing a common subscripts differ significantly ($P < 0.05$)

The results in Tables 24 and 25 indicate that after the rapid initial disappearance at the beginning of incubation there was only a very small further disappearance of nitrogen and dry matter.

There were significant ($P < 0.01$) interactions between formalin level and time for nitrogen disappearance and between sheep and formalin level for dry matter disappearance.

At the higher levels of formalin inclusion, the nitrogen and dry matter disappearance was decreased significantly ($P < 0.05$) by increasing the formalin level up to 40 cm^3 formalin kg^{-1} fish waste. There were small but significant ($P < 0.05$) increases in nitrogen disappearance associated with increasing time of incubation up to 3 hr only (see Table 29).

Effective degradability of nitrogen in fish silage

The data in Table 30 showing the (%) nitrogen disappearance from the bags (in Table 26) were subjected to the Ørskov and McDonald (1979) mathematical model to determine the effective degradability of nitrogen in the fish silage sample. The relationship between percentage nitrogen disappearance (P) and incubation time (t) has been shown to be exponential and can be described by the following equation:

$$P = a + b\,(1 - e^{-ct}).$$ (Ørskov and McDonald, 1979)

Where a, b and c are constants particular to each protein, (a) can be interpreted as a measure of the rapidly soluble protein fraction and (b) of the fraction which is subjected to degration; (c) being the rate constant of the degradation of (b) according to first order kinetics. The values of the constants a, b and c were derived from the incubation measurements by an iterative - least mean square curve fitting procedure. Assuming the passage rate from the rumen to be 0.05 hr^{-1} the effective protein degradability (dg) can be caluclated from the following relationship:

$$dg = a + \frac{bc}{c + k}$$ (Ørskov and McDonald, 1979)

Where k is the passage rate = 0.05 hr^{-1}

T A B L E   2 9

The effect of incubation time on (%) nitrogen disappearance from nylon bags of fish silage treated with high levels of[+] formalin

Incubation time (hr)	0*	3	6	8	16	24	SED ±
(%) Nitrogen disappearance from nylon bags	49.52[a]	55.35[b]	57.24[b]	55.45[b]	58.61[b]	58.61[b]	2.101

[+]Means not sharing a common subscripts differ significantly ($P < 0.05$)

*Washing machine only

The results of the calculation are shown in Table 30.

The results presented in Table 30 illustrate that the effective degradability (dg) was significantly ($P < 0.01$) lower with formalin treated fish silages at all levels of inclusion when compared with the untreated fish silage.

At lower levels of formalin inclusion there were no significant differences in effective degradability of nitrogen between treatments 2, 3 and 4. Significantly ($P < 0.05$) lower values of degradability were obtained with treatments 5 and 6.

At higher levels of formalin, the degradability values were decreased significantly by increasing the formalin level up to 40 cm^3 kg^{-1} fish waste (see Table 30). However, the inclusion of high level of formalin resulted in a semi-solid product which would be unsuitable for animal feeding. Analysis of variance on the data for nitrogen, dry matter disappearance (%) and effective degradability are given in Appendices 3, 4 and 5.

DISCUSSION

In this study the Dacron bag method described by Ørskov and Mehrez (1977) was used in an attempt to estimate the degradability of fish silage. This method is currently the best routine method available for estimating degradability with reasonable precision. A particular advantage of this technique lies in the ability to study the nutritive value of residual feed after rumen incubation in order to estimate the digestibility and utilisability of UDP passing to the duodenum (Mathers *et al.*, 1979).

Three sheep were used to estimate the degradability since a minimum of three has been found necessary to obtain an acceptable level of repeatability of measurement (Mehrez and Ørskov, 1977).

The results presented in Tables 26 and 25 clearly indicate that formaldehyde treatment of fish silage produced lower rates of disappearance of nitrogen and dry matter from nylon bags at all levels of application. The high levels of formalin treated fish silage gave lower values of (%) disappearance of nitrogen and dry matter when compared with the low levels, which produced values

TABLE 30

Estimates of effective degradability (%) of nitrogen from different types of fish silage at 0.05 hr^{-1} passage rate

Treatment No.	Formalin level (cm^3 kg^{-1} fish waste)	Effective degradability (dg)			Mean value of (dg)	SED ±
		Sheep 1	Sheep 2	Sheep 3		
1	0	100.0	100.0	100.0	100.0^a	0.447
2	1 (Low levels)	99.0	99.0	98.0	98.7^b	
3	2 (Low levels)	99.0	98.0	98.0	98.3^b	
4	3 (Low levels)	98.0	99.0	98.0	98.3^b	
5	4 (Low levels)	98.0	97.0	-	97.5^c	
6	5 (Low levels)	93.0	93.0	94.0	93.3^d	
7	0	100.0	100.0	100.0	100.0^a	3.50
8	10 (High levels)	63.0	60.0	59.0	60.7^b	
9	20 (High levels)	37.0	41.0	38.0	38.7^c	
10	30 (High levels)	39.0	44.0	42.0	41.7^c	
11	40 (High levels)	45.0	39.0	-	42.0^c	

*Means not sharing a common subscripts differ significantly ($P < 0.05$)

slightly lower than untreated fish silages.

The (%) disappearance of nitrogen and dry matter from nylon bags was variable in some cases even between bags incubated together in the same rumen at the same time. It seems that the variability recorded may have been caused by lack of homogenity of fish silage. Examination of the undigested portion of the silage which was left in the bag showed that the bags giving low (%) disappearances of nitrogen and dry matter contained more undissolved bones than the bags giving high (%) disappearances of nitrogen and dry matter. However, a high ash content in the sample could not have accounted for the high recovery of nitrogen. It was interesting to note that the rate of bag losses of nitrogen and dry matter of all types of fish silage was greater at the beginning of incubation. Increasing the time of incubation up to 21 or 24 hrs did not produce a large increase in (%) disappearance of nitrogen and dry matter.

The effective degradability values (dg) for all types of fish silage (see Table 30) were calculated from the rates of nitrogen disappearance from nylon bags using the Ørskov and McDonald (1979) model. It can be seen from Table 30 that formaldehyde treatment of fish silage reduced the effective degradability of fish silage nitrogen in the rumen in a pattern similar to that of (%) disappearance of nitrogen and dry matter.

The mean degradability values for the high levels formalin treatment (10, 20, 30 and 40 cm^3 formalin kg^{-1} fish waste) were 60.7, 38.7, 41.7 and 42.0 respectively, and for the low levels (1, 2, 3, 4 and 5 cm^3 formalin kg^{-1} fish waste) were 98.7, 98.3, 98.3, 97.5 and 93.3 respectively. The mean degradability value of both untreated silages (treatment 1 and 7) was 100. It appears from the results above that silages treated with the low levels of formalin were slightly less degradable than untreated fish silage.

The mean effect of formaldehyde on nitrogen disappearance from the nylon bag was probably due to its limiting effect of autolysis of fish protein in the silage. This leads to a more solid product which is retained for a longer time in the bags. At low or zero formaldehyde, a high proportion of fish silage nitrogen is in the form of soluble non-protein nitrogen (mainly amino acids) which would

leak rapidly from the bags (as confirmed by zero time incubations). In addition formaldehyde would be expected to exert a protecting effect on any residual true protein in the fish silage.

Appreciable quantities of residual nitrogen following rumen incubation were found only in the case of the high levels of formalin treatment (10, 20, 30 and 40 cm^3 formalin kg^{-1} fish waste) where the product was solid or semi-solid rather than liquid.

The bag technique is applicable only to solid protein feedstuffs because it assumes that nitrogen leaving the bag is completely degraded. Therefore, it cannot differentiate between solubilisation and degradation of the material contained in the bag. In this connection the work of Nugent and Mangan (1978) is relevant; these workers observed that the three soluble proteins, casein, fraction 1 leaf protein and bovine serum albumin were degraded at different rates by rumen micro-organisms. Findings of other workers also show that purified soluble proteins such as bovine serum albumin and ovalbumin are slowly degraded in vitro (Annison, 1956 and Mangan, 1972). Although less degradable, the fish silage produced using high formaldehyde levels was unsuitable for animal feeding, firstly because of its physical form which would make it difficult to handle and incorporate into dry diets ; secondly, the high levels of formalin would present a serious health hazard and would be likely to inhibit rumen microbial activity.

CONCLUSIONS

The results of this investigation showed that the in sacco technique was of little or no guide to degradability when applied to soluble protein sources such as fish silage, because it is based on the faulty assumption that all the material leaving the bag is completely degraded. This leads to over-estimation of degradability. Formaldehyde treatment of fish silage significantly reduced the losses of nitrogen and dry matter from the bags, but losses were very high with low levels of application. However, the differences were probably a reflection of the effect of formaldehyde on the physical form of the silage which would affect the amount of leakage from the bags. Therefore, there was need for further work to develop a method so that a reliable estimate of degradation rates of fish silage and the effect of formaldehyde addition could be obtained.

SECTION 3

DEVELOPMENT OF AN *IN VITRO* TECHNIQUE TO ESTIMATE FISH SILAGE PROTEIN DEGRADABILITY

A. A Study of Casein Degradation *in vitro*

OBJECTIVE

To investigate the reliability of the procedure used by Broderick (1978) to measure protein degradability using casein as a test protein.

EXPERIMENTAL

Animals

Two pairs of sheep, Suffolk cross and Scottish black face, fitted with permanent rumen cannulae were used to obtain rumen fluid. The animals were kept indoors during the experimental period and fed a maintenance diet of 800 g d^{-1} hay and 200 g d^{-1} concentrate given in two equal meals. The composition of the feeds is listed in Table 24.

Preparation for incubation

Rumen fluid was obtained from the donor sheep about 2 hr after feeding and transported immediately to the laboratory in a thermos flask. It was then strained through four layers of cheese cloth, maintained at 39°C and continuously gassed with carbon dioxide (CO_2) before use.

The strained ruminal fluid (SRF) was mixed with maltose as an energy source and dithiothreitol (DTT) as a reducing reagent to maintain anaerobic conditions. Maltose and DTT were dissolved in a small volume of McDougall's buffer (1948) (see Table 31) just prior to addition of SRF. Concentrations in the SRF were Maltose 93 m M and DTT, 1.6 m M respectively.

In initial incubations HCl-precipitated casein was used as the test protein. It was dissolved in McDougall's buffer by prolonged stirring (1-2 hr) on a magnetic stirrer to give a concentration of 4 mg cm^{-3}. Hydrazine sulphate (HS), an inhibitor of amino acid deamination and microbial ammonia uptake, was added to the McDougall's buffer (6 cm^3) to give a concentration of 2.67 m M.

Incubation

At the start of incubation, 10 cm^3 of SRF, containing added maltose and DTT, was added to 6 cm^3 of McDougall's buffer containing added

TABLE 31

Composition of artificial saliva*

Composition	Concentration (gl^{-1} distilled water)
$Na_2\ HPO_4\ 12H_2O$	46.50
$Na\ HCO_3$	49.00
$Na\ Cl$	2.94
$K\ Cl$	2.85
$Mg\ Cl_2\ 6H_2O$	0.64
$Ca\ Cl_2\ 6H_2O$	0.36

*McDougall (1948). Diluted 1 : 4 with distilled water before use in the procedure.

casein and HS. Final volume concentrations are shown in Table 32. Incubations were carried out in 100 cm^3 glass tubes. Following SRF addition, tubes were gassed with CO_2 and immediately stoppered with Bunsen valves and incubated at 39°C for 3 to 5 hr in a water bath. Incubation tubes were agitated by hand at 30 min intervals. Incubations were stopped at appropriate times (usually at 1 hr intervals) by adding 15 cm^3/tube uranyl acetate solution (9.6 g l^{-1}). Tubes were shaken and allowed to stand for 10 mins. at 0^oC then centrifuge for 10 mins. at 670 RCF.
The supernatant was carefully decanted into plastic containers and stored at 5°C before analysis of ammonia and total amino acids (see Chapter 2, Materials and Methods).

Analysis was carried out within 48 hr of completion of the incubation.

RESULTS

The initial part of the *in vitro* work examined the effect of hydrazine sulphate (HS) as an inhibitor of microbial uptake of amino acids and ammonia-N released during *in vitro* degradation of casein. Total accumulation of amino acids + ammonia in the presence of 1 m M HS was used as index of protein degradation. Identical amounts of casein (2.82 mg N) were used in each experiment and ammonia + amino acid-N was rapidly liberated in each instance, indicating the marked proteolytic activity of the strained rumen fluid. The dry matter and nitrogen content of the casein are shown in Table 33.

The degradation curve for casein (using means of five experiments) is shown in Figure 18. X is the natural logarithm of fraction of the original protein remaining undegraded. [Total casein-N added - (ammonia + amino acid-N)]. The curve was linear through 5 hr of incubation and had a Y-intercept of 0.237 which represents the fraction immediately degraded. A high correlation ($r = 0.940$) was observed.

The mean kd value (kd = fractional degradative rate constant = - slope of the log plot) from five individual experiments was 0.237 ± 0.021 (see Table 34). Thus 23.7% of the casein protein remaining at any time up to 5 hr incubation was degraded per hour.

Table 35 shows the proportion of ammonia and amino acid-N released from casein by incubation with SRF as a (%) of total casein-N added. As casein was degraded, in the presence of HS, the ammonia + amino acid-N concentrations increased with time to a maximum about 5 hr after the start of incubation, and was then equivalent to 73% of the

TABLE 32

a. Composition of the final incubation mixture in each tube

Composition	Volume (cm^3)
McDougall's buffer	6
Strained rumen fluid	10
Total	16

b. Concentrations at start of incubation in each tube

Material	Concentration
Maltose	58 m M
Hydrazine sulphate	1 m M
Dithiothretol	1 m M
Casein	1.25 mg cm^{-3}

TABLE 33

Mean dry matter, nitrogen and protein contents of feedstuffs tested

Feedstuff	Dry matter ($g\ kg^{-1}$)	Nitrogen ($g\ kg^{-1}$ DM)	Crude protein (N x 6.25) ($g\ kg^{-1}$ DM)
Casein	922	153	956
Soya bean meal	855	90	563
Fish meal	915	122	762
Bovine serum albumin	921	155	969
Pot ale	457	55	344

FIGURE18: LOG PLOT OF _IN VITRO_ CASEIN DEGRADATION IN THE PRESENCE OF HYDRAZINE SULPHATE. (Ln (x) = Log_e (TOTAL CASEIN-N ADDED-(AMMONIA + AMINO ACID-N))

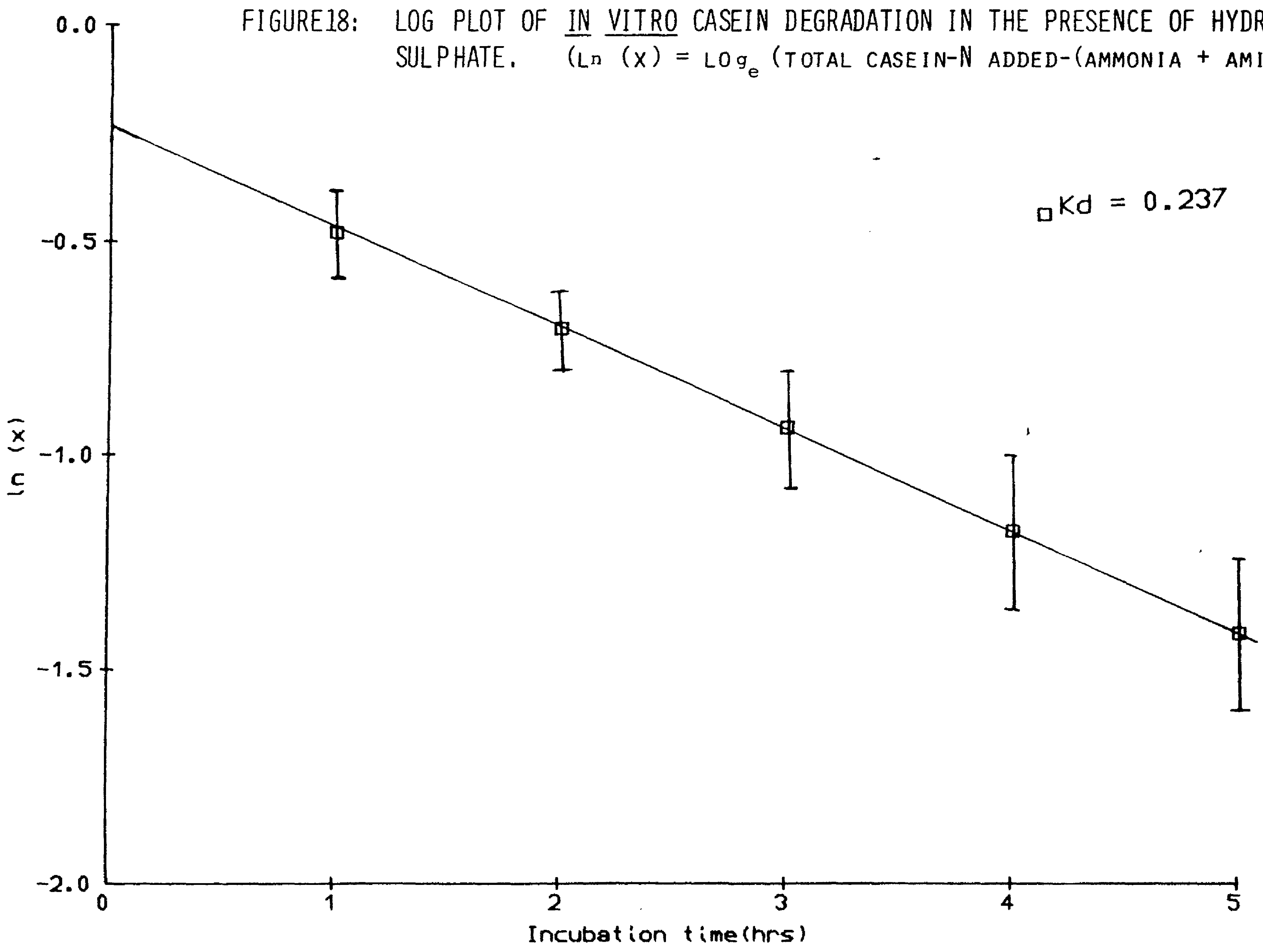


TABLE 34

In vitro kd values of casein in presence of 1 m M hydrazine sulphate (5 experiments)

Experiment No.	Fractional degradation rate constant (kd)
1	0.293
2	0.238
3	0.174
4	0.238
5	0.241
Mean	0.237
SE ±	0.021

TABLE 35

Mean recoveries of ammonia and amino acid-N (as a % of total casein-N added) from casein incubated in the presence or absence of hydrazine-sulphate (mean of 5 experiments)

Incubation time (hr)	+ HS						- HS	
	Ammonia-N		Amino acid-N		Ammonia + amino acid-N		Ammonia + amino acid-N	
1	5.58 SE	±0.90	34.95 SE	±5.26	40.53 SE	±4.09	22.89 SE	±3.66
2	7.34	±1.04	38.33	±2.20	45.66	±3.20	44.41	±2.42
3	9.20	±2.43	44.39	±5.46	53.59	±4.57	29.93	±3.73
4	11.00	±1.2	52.10	±4.84	63.10	±4.58	17.52	±1.12
5	13.31	±3.10	60.37	±2.60	73.31	±5.21	7.38	±0.69
	n = 5		n = 5		n = 5		n = 2	

total casein-N added. Under these experimental conditions only 13.3% of total casein-N appeared as ammonia-N, whilst about 60% was recovered as amino acid-N at the end of 5 hr incubation time.

In the absence of HS, the maximum levels of ammonia and amino acid were obtained after 2 hr of incubation. Thereafter, the levels decline and only 7% of the casein-N initially added was present as ammonia and amino acid after 5 hr (see Figure 19). The equation obtained by Broderick (1978) was used to calculate the proportion of dietary protein escaping ruminal degradation. The equation is:

$$\text{Estimated \% escape} = \frac{kr}{kr + kd} \times 100$$

Where,

kr = ruminal passage rate hr^{-1}

kd = fractional degradation rate constant.

The mathematical derivation of the above equation was described by Broderick (1978). Using a ruminal passage rate of 0.05 hr^{-1} (Ørskov and McDonald, 1979), the proportion of casein protein escaping the rumen and reaching the abomasum undegraded can be estimated for this experiment using the above equation as follows:

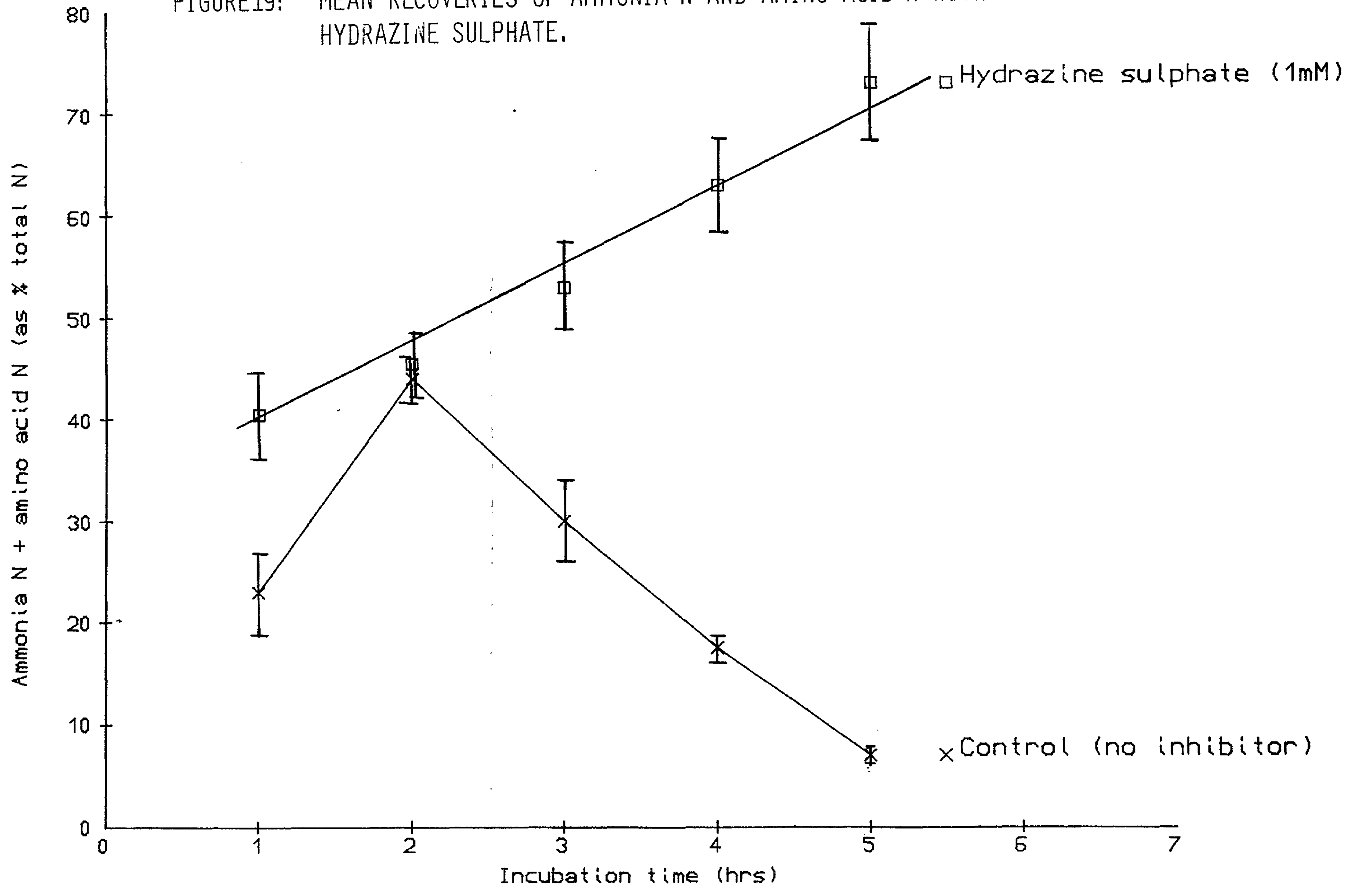
$$\text{Estimated \% escape} = \frac{0.05}{0.05 + 0.237} \times 100$$

= 17.4% nitrogen would be expected to pass out the rumen undegraded. Thus the effective degradability of casein would be 83% at a ruminal passage rate of 0.05 hr^{-1}.

DISCUSSION

The increased knowledge of nitrogen metabolism in the ruminant collated by the ARC (1980) provided the framework for a new system of protein rationing which has now been adopted by a number of organisations. In this new system, the degradability of feed proteins assumes an important and central role since the microbes degrade dietary proteins to varying degrees affecting the quantity and quality of the amino acids eventually absorbed by the host animal (Mohammed and Smith, 1977). Thus the estimation of degradability of the different types of fish silage produced was important in this work to help with the selection of the best silage for use as a

FIGURE19: MEAN RECOVERIES OF AMMONIA-N AND AMINO ACID-N WITH OR WITHOU HYDRAZINE SULPHATE.



protein supplement in the calf feeding trial.

Current methods of estimating dietary protein degradability are expensive, time consuming and involve the use of surgically modified animals (see Chapter 1, Section 1). This situation is clearly unsatisfactory, particularly if routine analysis of feedstuffs is to be undertaken. *In vitro* methods not only have the advantage of being less expensive and less time consuming, but also give the chance to maintain experimental conditions more precisely than *in vivo* trials permit. An *in vitro* method (Broderick, 1978) for estimating protein degradability has been examined, using casein and different feed proteins (see Section-3B).The method involves the use of hydrazine sulphate (HS) to inhibit re-utilisation of amino acids and ammonia released by proteolysis with rumen fluid. Hydrazine is well known to be an inhibitor of vitamin B -dependent enzymes and hence of amino acid metabolism (Amenta and Johnston, 1963 and Broderick, 1978). It inhibits by reacting with the carbonyl group of pyridoxal and pyridoxal phosphate (Sanberlich, 1968, cited in Broderick and Balthrop, 1979). This inhibitor also inhibits ammonia utilisation by inhibiting glutamic dehydrogenase, thought to be a major enzyme of microbial ammonia fixation. The rate of degradation was determined by the increase in concentrations of ammonia and amino acid-N.

In vitro degradation of casein

The degradation of casein was studied in five experiments using the *in vitro* procedure described earlier. The mean degradative rate constant (kd) observed from the five individual experiments was 0.237 hr^{-1} (SE ±0.021). Using a ruminal passage rate (kr) of 0.05 hr^{-1}, the proportion of casein that would escape degradation in the rumen was estimated to be 17%.

This value is in reasonable agreement with published *in vitro* and *in vivo* degradabilities obtained by Broderick (1978); Mahadevan *et al*. (1979); Raab *et al*. (1983) and McDonald and Hall (1957) (see Table 36).

Low ratios of test protein to SRF were used (2 mg casein cm^{-3} SRF - approximately equivalent to those expected *in vivo*) so that the

TABLE 36

Degradability values of casein reported in the literature

Author	Method used	Incubation time used (hr)	Degradability (%)
Broderick (1978)	*in vitro*	3	85.8
Mahadevan *et al*. (1979)	*in vitro*	18	90.0
Raab *et al*. (1983)	*in vitro*	24	99.0
McDonald and Hall (1957)	*in vivo*	8	90.0
In this study	*in vitro*	5	83.0

accumulated end products of fermentation would not inhibit degradation. This would be particularly likely for the fourth and fifth hour of incubation where microbial numbers are fewer and end products could occupy a larger proportion of enzyme active sites (Broderick, 1978). Thus, unless care is taken, end product inhibition is often the reason that *in vitro* degradation does not completely reflect degradation *in vivo*. However, the above reason could explain the slightly lower degradability measured in this study (see Table 36).

The slight variations in degradative rate values (kd) listed in Table 34 could be attributed to the fluctuation in degradative activity of SRF batches obtained on different days.

Table 35 clearly shows that in the absence of inhibitor (control), casein is readily attacked with the liberation of amino acids and ammonia. However, the rise in amino acid and ammonia was of brief duration and after 2 hr the value began to decline sharply (see Figure 19).

In contrast, in the presence of HS,levels of ammonia and amino acids rose quickly and continued to rise for 5 hr. Recoveries of amino acid and ammonia-N after 5 hr incubation with HS (see Table 35) showed that amino acid production was about 5 times that of ammonia. This result agrees with the finding of Broderick (1978) who reported a similar pattern of amino acid and ammonia release. The differences in recoveries between amino acids and ammonia can be explained by the inhibition of the deamination power of the organisms due to the addition of HS. At the concentration tested, HS did not appear to depress casein degradation *in vitro*.

The rapid degradation of casein protein can be attributed to the absence of disulphide bonds, and the helical structure of casein (Mercier *et al.*, 1972). It appears possible in many feedstuff proteins to predict from the disulphide content of the protein the degree of susceptibility to degradation by rumen bacterial-proteases (Mangan, 1972 and Mahadevan *et al.*, 1980).

Some details of the incubation technique are discussed below:

a. The use of maltose and DTT

The reason for including maltose and DTT in the inoculum was respectively to supply the rumen organisms with a readily available source of energy and to maintain better anaerobic conditions during incubation. This ensures greater and more reliable degradation *in vitro* (Broderick, 1978).

b. Substrate level used

A substrate level of 20 mg casein (2.82 mg N) per 10 cm^3 SRF was found to give relatively high degradation activity for periods of up to 5 hr incubation. This level (2.82 mg N) was also routinely used for feedstuff proteins in later experiments.

c. The use of sufficient blanks

Adequate controls are essential in this type of experiments and in each case analysis was made on inoculum without casein (substrate) and appropriate corrections made to the experimental results.

d. Optimum pH

Variation of the pH of the incubation media was found to have little effect on proteolytic activity within the range of pH 6 to pH 7 (Blackburn and Hobson, 1960 and Borchers, 1965).

e. Reliability of ammonia and amino acid assay

The experimental results in both assays showed reasonable accuracy. However, it is worth noting that the phenate-hypochlorate reagents and casein hydrolysate standards were not stable after storage and had to be prepared fresh daily.

f. Rumen fluid

The use of rumen fluid with minimum dilution (10 cm^3 SRF + 5 cm^3 McDougall's buffer) was desirable to produce higher proteolytic activity. Active degradation was achieved with casein and other feedstuff proteins using 2:1 dilution of the strained rumen fluid.

B. A Comparison of Protein Degradation of a range of Feedstuffs

OBJECTIVE

The aim of this experiment was to apply the *in vitro* procedure previously described in Section 3A to various feedstuff proteins including fish silage.

EXPERIMENTAL

The breakdown of untreated fish silage, formalin-treated fish silage (5 cm^3 formalin kg^{-1} fish waste), bovine serum albumin (BSA), soya bean meal, white fish meal and pot ale proteins by SRF was examined. The six proteins were incubated using the previous *in vitro* method (see Section 3A). The soluble proteins (untreated fish silage, BSA and pot ale) were dissolved in 6 cm^3 McDougall's buffer, but the partially soluble proteins (formalin-treated fish silage, soya bean meal and white fish meal) were suspended in McDougall's buffer. Duplicate samples of each protein (2.82 mg-N each) were incubated for 4 to 5 hr with incubations stopped every hour and the supernatant of each digest was analysed for ammonia-N and total amino acids.

RESULTS

The validity of the Broderick *in vitro* method was tested by comparing the values it produced for a range of feedstuffs with those obtained by other methods.

The mean degradative rate constant (kd) and estimate of the proportion of protein escaping ruminal degradation for each feed protein is shown in Table 37. The value used for passage rate was 0.05 hr^{-1}. Dry matter and nitrogen contents of feedstuffs are shown in Table 33.

DISCUSSION

We assumed that all of the six feed protein tested were degraded in a manner similar to casein (e.g. essentially as one protein fraction degraded at a single rate). However, the results with pot ale and

TABLE 37

Mean estimates of *in vitro* degradability of different protein sources after 5 hr incubation in the presence of hydrazine sulphate

Feedstuff	Degradative rate (kd) hr^{-1}	Estimated* (%) escape	Effective* degradability (%)
Bovine serum albumin	0.004	92.59	7.41
White fish meal	0.028	64.10	36.90
Soya bean meal	0.071	41.32	58.68
Pot ale	0.088	36.23	63.77
Formalin-treated fish silage	0.0030	94.34	5.66
Untreated fish silage	0.0180	73.53	26.47
Casein (value obtained from Section-3A)	0.237	17.4	82.6

*Calculated using a rumen passage rate of 0.05 hr^{-1}

fish meal were not consistent with this interpretation. Degradation rates for pot ale samples were as high as for casein in the first 2 hr of incubation but very slow after that. The results suggest that there were at least two pot ale protein fractions degraded at different rates. The rapid degradation rate observed initially would be principally due to the more rapidly degraded fraction.

Incubation of white fish meal produced a slow increase in the concentrations of ammonia-N and amino acid-N during the first 2 hr, but no further increase occurred during the remaining incubation. This reflected the resistant properties of white fish meal protein to proteolysis due to the heat treatment during manufacture.

Soya bean meal was clearly more degradable than white fish meal and BSA at all intervals of incubation (see Table 37). However, there was a marked decrease in degradation towards the end of the incubation due possibly to end products inhibition of proteolysis.

Bovine serum albumin was only slightly degraded on incubation with SRF. This soluble protein was degraded at less than quarter the rate for casein. Resistance of BSA to degradation has been noted *in vivo* (Annison, 1956) and *in vitro* using a crude microbial fraction (Mahadevan *et al.*, 1980). The latter workers suggested that the resistance to proteolysis of BSA is due to its tightly folded globular structure cross linked with disulphide bridges.

The method failed to estimate satisfactorily the degradation rate of both fish silages (untreated and formalin-treated fish silages) since very low estimates of kd were observed (see Table 37). This is because the method assumes that all protein measured as amino acids is totally degraded. Whilst this may be an acceptable approximation for typical feed proteins, it will not be the case for soluble liquid feed proteins such as fish silage which contains high initial levels of free amino acids and might have a rapid rumen passage rate. The slow release of amino acids observed with formalin-treated fish silage indicates that the insoluble protein in fish silage is resistant to proteolysis.

The results show that the *in vitro* method tested is capable of ranking certain feed protein degradabilities in a similar order to

that observed by other workers using other methods (see Table 38). The method was capable of measuring degradation of feed proteins ranging from as low as 7 to 83%. However, the method required modification before it could be applied to more complex feedstuffs containing proteins of varying solubility and degradability, as otherwise only the more rapidly degradable components would be determined. The work of Broderick and Craig (1980) is relevant. They developed a new mathematical model suitable for estimating degradability for proteins containing two protein fractions degraded at two different rates after *in vitro* incubation using the same method.

The method has been shown to be unsuitable for proteins containing high initial levels of amino-acids as it considers all amino acids to be degraded in the rumen. Thus, it failed to describe the rate of amino acid degradation in fish silage which is of major importance in its ruminal nitrogen metabolism.

CONCLUSIONS

Results of the inital experiments conducted to estimate the rate of protein degradation using the *in vitro* method of Broderick (1978) suggested that hydrazine sulphate strongly inhibits amino acid degradation and the assimilation of ammonia-N by rumen micro-organisms. The technique proved easy and provided measurements of degradability. However, the method was unsuitable for estimating the rate of degradation of fish silage since amino acids deamination was almost totally inhibited. An inhibitor is required which allows measurement of ammonia-N accumulation from fish silage without interference from variable assimilation by micro-organisms and which includes ammonia-N from amino acid deamination. This would be a reliable index of the effect of formaldehyde treatment on the *in vitro* degradation of fish silage. Therefore, we became interested in the mechanism of ammonia-N fixation by rumen micro-organisms and in testing alternative chemical inhibitors to HS.

TABLE 38

The degradability of feedstuffs protein tested as reported in the literature

Feed protein	Effective degradability (%)	Method used	Author
Fish meal	38.0	*in sacco*	Miller and Laycock (1983)
	30.0	*in sacco*	Cronje and Mackie (1983)
	29.0	*in vitro*	Miller (1978)
Soya bean meal	56.0	fungal protease	Laycock and Miller (1983)
	70.0	*in sacco*	Laycock and Miller (1983)
	69.9	*in vitro* rumen fluid	Laycock and Miller (1983)
Bovine serum albumin	3.1	*in vitro* crude microbial fraction	Mahadevan *et al.* (1979)

C. Modification of the *in vitro* Technique to Measure Ammonia-N Release from Fish Silage Protein

OBJECTIVE

The initial phase of this investigation was concerned with the establishment of an *in vitro* incubation procedure as an index of protein degradation. This aim was achieved by applying the technique of Broderick (1978), but analysis of the nitrogenous end products of incubation showed that ammonia-N was the minor component and that amino acid-N made up approximately 80% of the total N released. It was considered that this would make the method unsuitable for the evaluation of fish silage in which amino acids and small peptides form a high proportion of the substrate initially present.

Alternative inhibitors to hydrazine sulphate were tested to find one which would maximise ammonia production *in vitro* and allow the assessment of degradation to be based solely on ammonia-N accumulation. In the absence of inhibitor ammonia-N was utilised rapidly by rumen micro-organisms for microbial protein synthesis and does not accumulate during incubation.

Inhibitors tested

The chemicals used as inhibitors of ruminal ammonia uptake were penicillin-G (procain salt), glutaric acid, m-bromobenzoic acid and hydrazine sulphate, all potential inhibitors of glutamic dehydrogenase. The inhibitors concentrations used are shown in Table 39.

TABLE 39

Concentration of the various inhibitors tested

Inhibitor	Concentration
Penicillin-G	100 IU
Glutaric acid	20 m M
m-Bromabenzoic acid	20 m M
Hydrazine sulphate	1 m M

EXPERIMENTAL

The same in vitro technique was used as described earlier in Section 3A using casein as the test protein (20 mg casein/tube) with the following two modifications when HS was replaced by other inhibitors:

1. The inoculum contained 2 m M dithiothreitol (DTT) to maintain better anaerobic conditions.

2. The inoculum contained no maltose since Broderick (1978) reported that all the inhibitors used, apart from HS, had no apparent effect on uptake of ammonia-N in the presence of readily fermentable carbohydrates.

The various inhibitors (at appropriate concentration) were pipetted into incubation tubes as solutions in 1 cm^3 McDougall's buffer. Those inhibitors which were only sparingly soluble in aqueous media (Penicillin-G and m-bromobenzoic acid) were suspended in the 6 cm^3 McDougall's buffer (also containing the casein) with continuous mixing and rapidly dispensed to incubation tubes. All incubations were stopped 3 hr after the addition of SRF, and ammonia-N recoveries were measured (sample minus blank concentration). Incubation was conducted in triplicate with the same batches of SRF for each experiment.

RESULTS

Results of this experiment are reported in Table 40. Initial experiments comparing Penicillin-G, glutaric acid, m-bromobenzoic acid and hydrazine sulphate showed that the highest recoveries of ammonia-N (as % of total-N added) were obtained with glutaric acid (15%) and Penicillin-G (13.8%), while only 8% was recovered in the HS incubations. Again the rate of liberation of amino acid-N with HS exceeded that of ammonia-N with a ratio of 1:8 (ammonia-N : amino acid-N). The ratios in the case of glutaric acid and Penicillin-G incubations were 1:2.2 and 2.3 respectively. No results were obtained with m-bromobenzoic acid because of its high insolubility in aqueous media and its interference with the analytical methods.

The effect of glutaric acid (20 m M) and HS (1 m M) on in vitro ammonia release was also studied in a series of 5 hr incubations in which casein was used as a substrate. The results are shown in

TABLE 40

Effect of hydrazine sulphate or glutaric acid on ruminal ammonia-N release from casein incubated *in vitro*

Inhibitor used	Concentration used	Incubation time (hr)	Ammonia released as % of total-N incubated		Mean	Ammonia-N: amino acid-N ratio
			Experiment 1	Experiment 2		
Hydrazine-sulphate	1 m M	3	8.0	8.0	8.0	1:8
Glutaric-acid	20 m M	3	17.0	13.0	15.0	1:2.2
Penicillin-G	100 IU	3	16.0	11.5	13.8	1:2.3

Table 41. Recoveries of ammonia-N (as % total-N added) after 5 hr incubation in the presence of glutaric acid were 30% while only 11% ammonia-N was recovered using HS (see Figure 20). Both recoveries were greater than that of controls (see Table 41 and Figure 20).

From these results it seems that glutaric acid was the most effective inhibitor of ammonia uptake giving the highest ammonia-N recoveries.

TABLE 41

Effect of hydrazine sulphate and glutaric acid on the recovery of ammonia-N as % of total casein-N incubated *in vitro*

Incubation time/hr	Glutaric acid (20 m M)		Hydrazine sulphate (1 m M)		Control (no inhibitor)	
1	10.25 SE	1.25	5.36 SE	0.85	15.50 SE	2.12
2	14.50	0.50	6.64	0.85	24.83	2.04
3	20.50	1.50	8.24	1.25	19.00	1.27
4	24.00	1.00	9.55	2.10	15.50	1.57
5	29.85	1.15	11.20	1.95	6.06	1.65
	n = 2		n = 2		n = 2	

T A B L E 4 2

Ammonia-N production *in vitro* from untreated and formalin treated fish silages during 5 hr incubations in the presence of glutaric acid

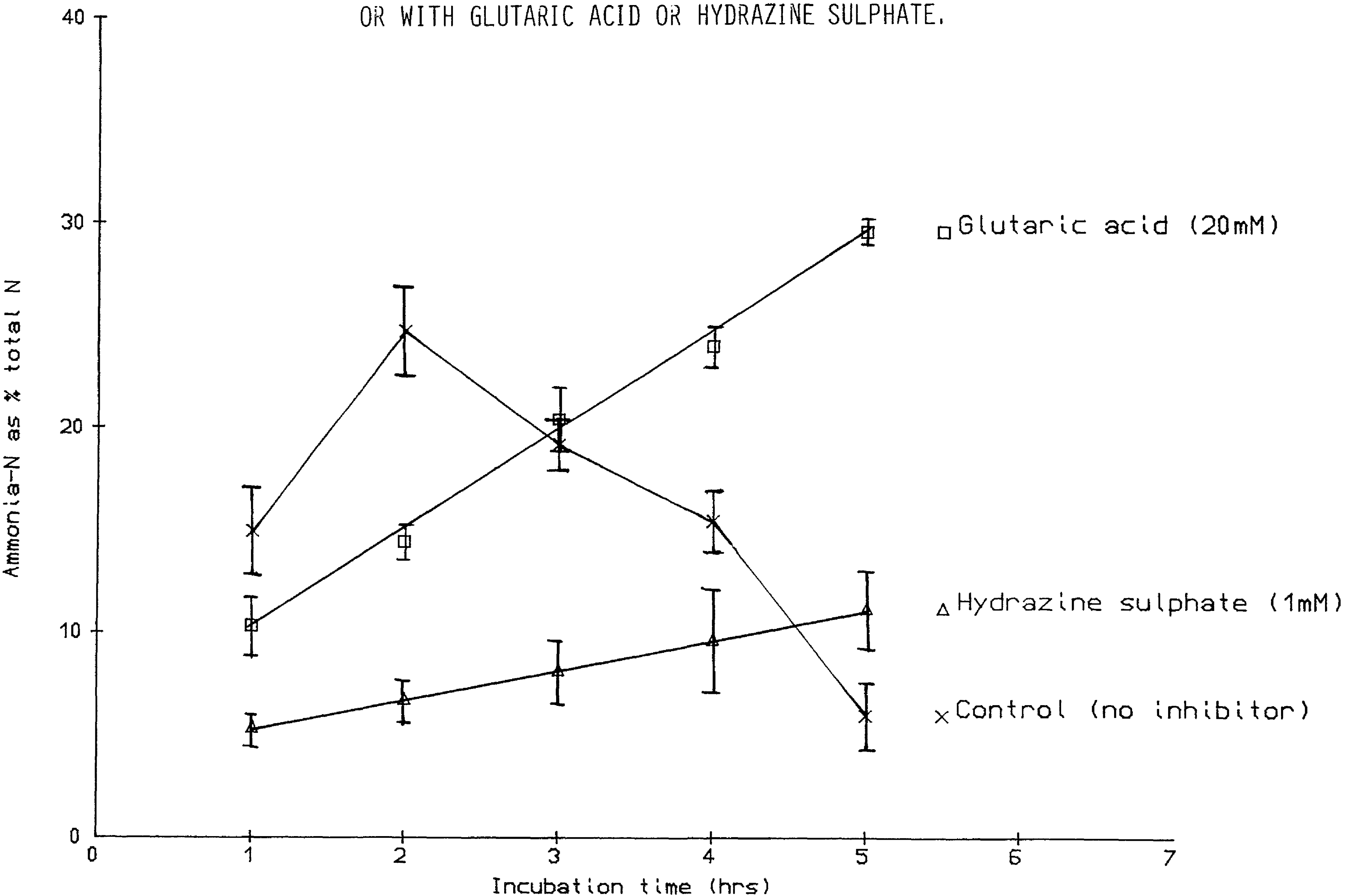
Treat -ment No.	Formalin level (cm^{-3} kg^{-1} fish waste)	Ammonia production as % of total-N incubated: Incubation time (hr) 0	1	2	3	4	5	Mean kNH_3* hr^{-1}	Ammonia-N production as % of untreated control after 5hr incubation
1	0	10.00 SE ±0.495	14.15 SE ±1.485	19.10 SE ±1.536	26.65 SE ±0.636	33.90 SE ±0.141	39.70 SE ±0.990	6.10[a] SE ±0.420	100.0
2	1	4.97 ±0.013	11.13 ±0.250	22.26 ±1.776	24.31 ±2.193	30.69 ±3.908	34.54 ±0.971	5.96 ±0.240	87.0
3	2	6.42 ±1.342	14.92 ±0.514	18.45 ±0.636	20.84 ±0.345	27.32 ±1.153	ND[+]	4.76 ±0.127	80.6
4	3	7.00 ±0.848	12.65 ±0.636	16.00 ±0.141	21.15 ±1.343	25.90 ±2.121	30.70 ±1.697	4.64 ±0.494	77.3
5	4	7.20 ±0.424	10.8, ±0.989	13.5, ±0.410	18.90 ±1.697	20.9, ±0.989	25.0, ±0.566	3.20 ±0.608	62.9
6	5	6.85 ±1.768	11.20 ±0.566	12.70 ±0.283	17.70 ±2.404	18.75 ±1.344	23.40 ±0.0	3.15[b] ±0.487	58.9

*kNH_3 = The rate of ammonia-N production (% added N recovered as ammonia per hour)

[+]Not determined

[x] Means not sharing common subscripts differ significantly ($P < 0.05$)

FIGURE 20: AMMONIA-N FORMATION *IN VITRO* FROM CASEIN INCUBATED EITHER WITHOUT INHIBITOR OR WITH GLUTARIC ACID OR HYDRAZINE SULPHATE.



DISCUSSION

The experiment studied the effect of the various chemicals listed in Table 39 on the recovery of total-N incubated as ammonia-N. Among the inhibitors examined were hydrazine sulphate (Amenta and Johnston, 1963 and Broderick, 1978), Penicillin-G (Lewis and Emery, 1962), glutaric-acid and m-bromobenzoic acid (Caughey *et al*., 1957). The effectiveness was assessed from the level of ammonia-N recovery.

The results showed that the highest ammonia-N recoveries were obtained with glutaric acid and Penicillin-G. The results for Penicillin-G are in contast with the findings of Broderick and Balthrop (1979). They reported that Penicillin-G gave recoveries (ammonia-N + amino acid-N) no different from control (without inhibitor). The higher recoveries obtained in this experiment are attributed to the high concentration of Penicillin-G used (100 IU/tube) which is double the concentration they used (50 IU/tube). However, Penicillin-G was less convenient than glutaric acid because of its insolubility. The initial incubations with m-bromobenzoic-acid failed because it interfered with subsequent assays for amino acid and ammonia.

The reason for excluding maltose from incubations (apart from HS incubations) was because the presence of readily available sources of energy enables the microbes to use the ammonia-N released as a source of nitrogen for growth and may cause a steady decline in the concentration of ammonia-N even in the presence of glutaric acid. The higher DTT concentration used was necessary to maintain better anaerobic conditions in the absence of maltose.

Further incubations were conducted to compare HS with glutaric acid as inhibitors of ammonia-N uptake. When HS was used, total ammonia-N recoveries generally confirm the results observed by Broderick (1978) and Broderick and Craig (1980) and the results of the initial experiment reported in Section-3A. Recoveries of ammonia-N in the presence of HS (1 m M) are lower than for glutaric acid (20 m M) because the former inhibits deamination more effectively. Thus incubation with HS resulted in lower recoveries of ammonia-N but higher recoveries of amino acid-N (ammonia-N:amino acid-N = 1:8). At concentrations tested recoveries of ammonia-N with glutaric acid

were almost three times that obtained with HS. These results indicate that glutaric acid may be potentially useful for reducing ruminal ammonia-N uptake by micro-organisms without totally inhibiting amino acid deamination. Concentration of 20 m M was sufficient to arrest ammonia-N uptake by microbes to a large extent. HS was found to have a strong inhibiting effect on amino acid deamination and cannot be used to study ammonia-N release *in vitro.*

CONCLUSIONS

Glutaric acid gave the most favourable response in this study because it gave the highest ammonia-N recoveries and was convenient (cheap, soluble and did not interfere with subsequent analysis). It was decided to use glutaric acid as an inhibitor of ammonia-N uptake in subsequent work to study the effect of formaldehyde treatment of fish silage on its ruminal proteolysis.

SECTION 4

MEASUREMENT OF IN VITRO AMMONIA PRODUCTION FROM FISH SILAGE PROTEIN AND THE EFFECT OF FORMALDEHYDE TREATMENT

OBJECTIVE

The aim of this experiment was to obtain information about the degradation of untreated fish silage and to evaluate the effect of treating fish silage with various levels of formaldehyde. Ammonia-N production *in vitro* was used as an index of rumen microbial degradation. The results were used in planning of the calf feeding trial.

EXPERIMENTAL

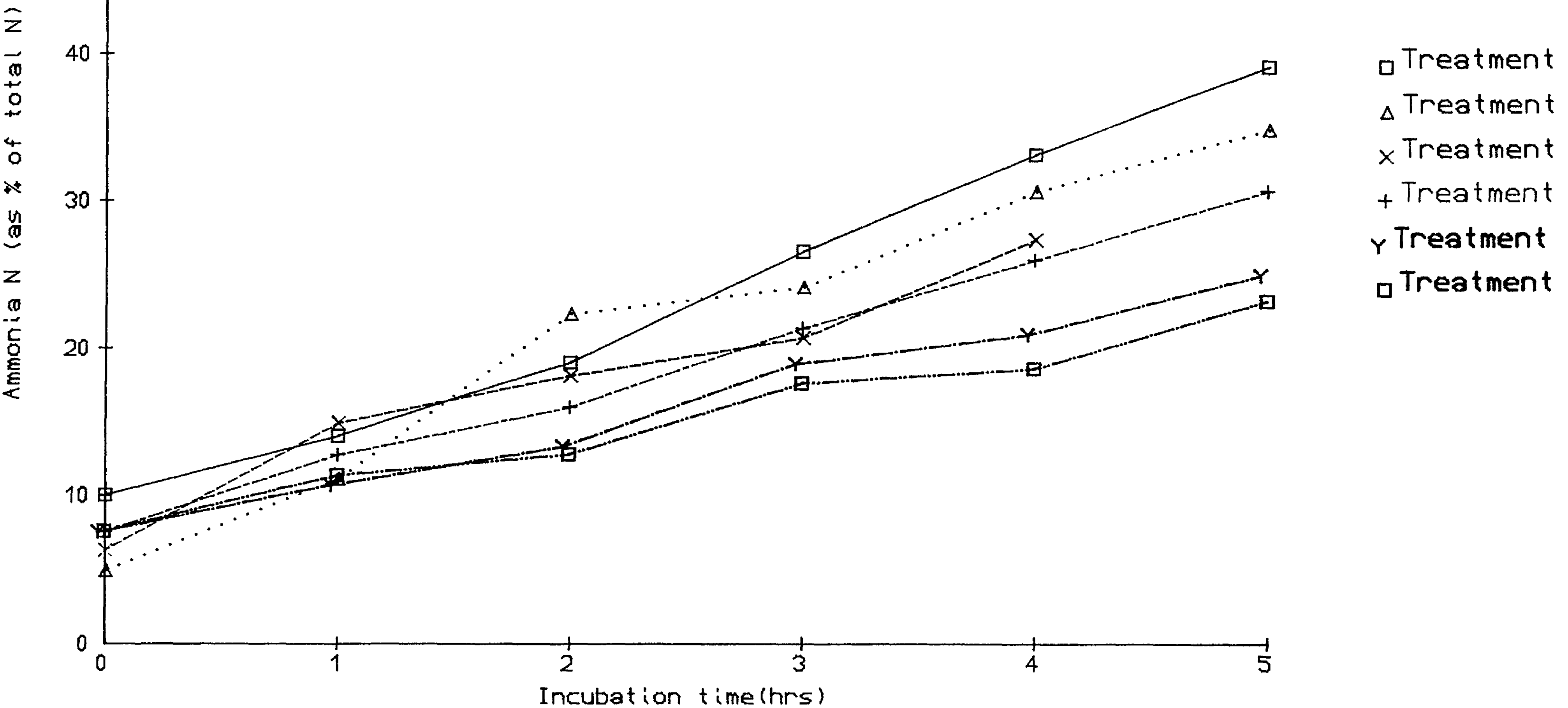
The experiment was conducted to compare ammonia-N production from fish silage treated with various levels of formalin (1, 2, 3, 4 and 5 cm^3 formalin kg^{-1} fish waste), using the *in vitro* procedure described in Section - 3C. Ammonia-N formation was measured over 5 hr incubation in buffered ruminal fluid in the presence of glutaric acid (20 m M) as an inhibitor of ammonia-N uptake. Duplicate samples of well homogenised fish silage (see Table 20 for treatment details) and sample of casein, white fish meal and urea (containing about 2.82 mg-N) were incubated for up to 5 hr, with incubations being stopped every hour. The supernatant from each digest was analysed for ammonia-N using the phenate-hypochlorate procedure.

RESULTS

In vitro ammonia production from fish silage protein

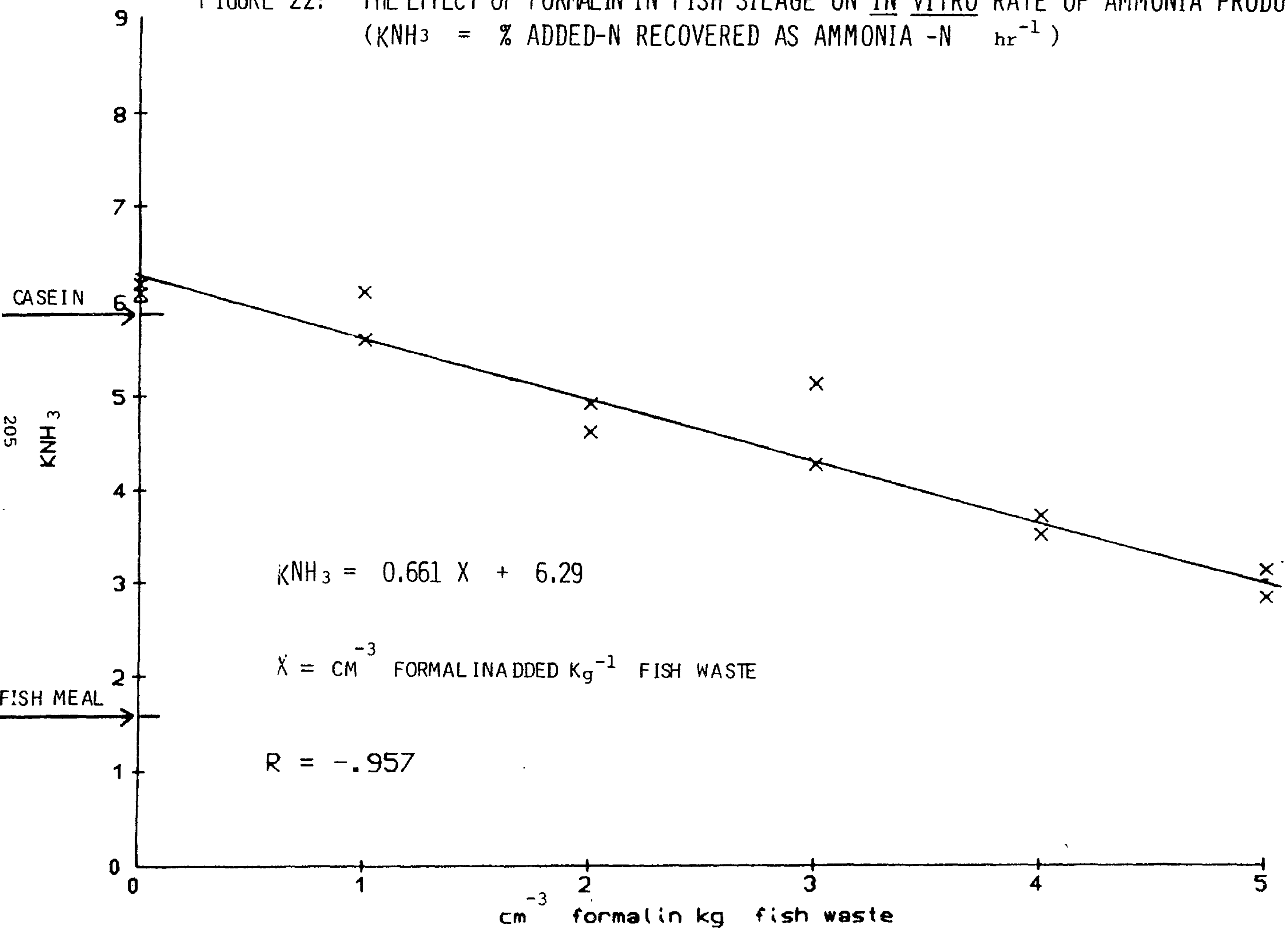
Ammonia-N production from fish silages treated with different levels of formalin is shown in Table 42 and Figure 21. *In vitro* ammonia-N production after 5 hr incubation for formalin treated silages (expressed as % of the values for their untreated controls) is also presented in Table 42. Ammonia-N production from fish silage treated with 1, 2, 3, 4 and 5 cm^3 formalin kg^{-1} fish waste were 87%, 80.6%, 77.3%, 62.9% and 58.9% of the value for untreated fish silage (control) respectively. The effect of formalin level on rate of ammonia-N production per hour (kNH_3) is shown in Table 42 and Figure 22. Ammonia release after 5 hr incubation was significantly related to formalin level (r = 0.96).

FIGURE 21: AMMONIA-N PRODUCTION FROM UNTREATED AND FORMALIN TREATED FISH-SILAGES *IN VITRO*.



*SEE TABLE 20 FOR TREATMENT DETAILS

FIGURE 22: THE EFFECT OF FORMALIN IN FISH SILAGE ON *IN VITRO* RATE OF AMMONIA PRODUCTION. (KNH_3 = % ADDED-N RECOVERED AS AMMONIA -N hr^{-1})



Six determinations of kNH_3 for the control (treatment 1) and for 5 cm^3 formalin (treatment 6) were carried out. Analysis of variance showed that formalin significantly ($P < 0.01$) reduced the rate of ammonia-N production. The highest level of formalin (5 cm^3 kg^{-1} waste) led to the lowest rate of ammonia-N production compared to the other treatment. There were significant ($P < 0.01$) increases in ammonia-N production associated with increasing time up to 5 hr of incubation. There was a small but significant ($P < 0.05$) interaction between time and formalin level.

Analyses of variance on (kNH_3) values and the rate of ammonia-N production are given in Appendices 6 and 7.

In vitro ammonia-N production for different feed proteins

The object of this comparison was to compare the rates of proteolysis of untreated fish silage, formalin treated fish silage, white fish meal, casein and urea. The results which are presented in Table 43 show that their rates of *in vitro* ammonia-N production per hour (kNH_3) were markedly different. After 5 hr incubation the $k NH_3$ for urea was significantly ($P < 0.05$) higher than that of other protein sources. The total ammonia-N production with urea reached its peak concentration after only 1 hr incubation (see Figure 23).

The rate of ammonia-N production from white fish meal protein was significantly ($P < 0.05$) lower than for the other proteins tested. The kNH_3 value of formalin treated fish silage was intermediate between the values of white fish meal and casein, and significantly ($P < 0.05$) lower than that of untreated fish silage. Untreated fish silage protein was rapidly degraded and the value of kNH_3 measured was significantly ($P < 0.05$) higher than that obtained with formalin treated fish silage, white fish meal and even casein (not significant).

DISCUSSION

Formaldehyde treatment of fish waste

The discovery by McDonald (1948) that soluble dietary proteins are extensively degraded to ammonia-N in the rumen and the subsequent observation that proteins or amino acids administered post-ruminally resulted in greater nitrogen retention than those added directly

TABLE 43

The mean[x] rates of *in vitro* ammonia production per hour (kNH_3) for different feed proteins during 5 hr incubations in the presence of glutaric acid

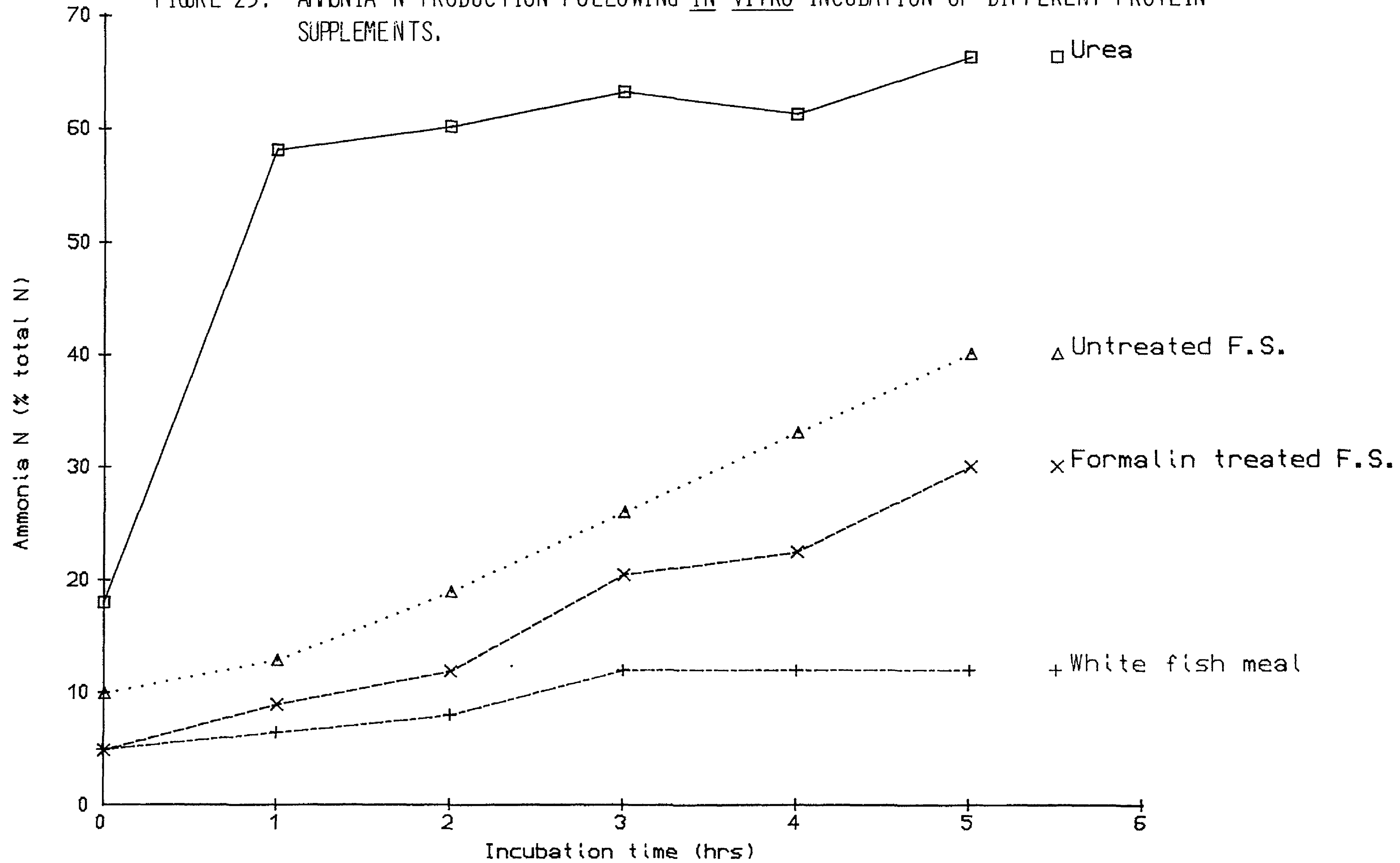
Object	Feed proteins tested				
	Fish meal	Formalin treated fish silage	Untreated fish silage	Casein	Urea
kNH_3 n = 12	1.60^{a} SD* ±0.26	4.29^{b} SD ±0.29	6.10^{c} SD ±0.25	5.80^{c} SD ±0.40	40.24^{d+} SD ±1.06

[x] Means not sharing common subscripts differ significantly ($P < 0.05$)

*SD of gradient of regression line (excluding urea)

[+] The rate was calculated for 1 hr only

FIGURE 23: AMMONIA-N PRODUCTION FOLLOWING *IN VITRO* INCUBATION OF DIFFERENT PROTEIN SUPPLEMENTS.



*F.S. = FISH SILAGE

into the rumen (Schelling and Hatfield, 1968) has led to attempts to find ways of protecting high quality dietary proteins from microbial degradation within the rumen. Treatment with formaldehyde appeared to be a potential method of decreasing rumen degradation of high quality protein and allowing more dietary protein to by-pass the rumen to the abomasum and lower digestive tract (Barry, 1976; Ferguson, 1975 and Offer, 1973). However, increased passage to the duodenum does not necessarily mean that the extra protein is digested efficiently nor, if digested, that the resultant profile of absorbed amino acids is such that it provides a better balance of amino acid for production (Ashes, *et al.*, 1984).

The object of this work was to determine the effect of treating fish silage with varying levels of formalin (as 40% formaldehyde) upon *in vitro* ammonia-N production. The degree of reduction in ammonia-N formation *in vitro* brought about by the protective agent (formalin) was used as the index for estimating its effectiveness *in vitro* in the rumen.

The results showed that untreated fish silage gave high rates of ammonia-N production which indicates that the soluble amino acids and peptides, which make up most of its N, were rapidly deaminated by rumen micro-organisms. The results presented in Table 42 clearly illustrate that formalin treated fish silages were deaminated to a lesser extent than the untreated control. This suggests that the formalin treated fish silage would be more resistant to microbial degradation in the rumen and could potentially lead to higher efficiency of utilisation of treated fish silage protein by increasing the supply of amino acid to the duodenum. Further, the amino acid composition of fish silage is such that a high nutritional value of rumen by-pass protein would be expected (Gildberg and Raa, 1977 and Strøm and Eggum, 1981) and thus the nutritional advantage from limiting proteolysis in the rumen could be considerable.

The rate of degradation was high for untreated fish silage protein indicating that only a small proportion would excape the rumen undegraded. Reducing the rate of ammonia-N production would also lead to a reduction in the loss of ammonia-N to the blood and may improve the efficiency of capture and released ammonia-N by rumen micro-organisms. Therefore, it is important to attempt to improve

its nutritional value of fish silage by formaldehyde treatment.

The lower rate of ammonia-N release from formalin-treated fish silages may indicate that formalin affects the availability of treated fish silage proteins to the rumen micro-organisms. Similarly formalin inhibits the enzymatic degradation of fish protein during fish silage processing (see Section 1 of this Chapter). The results obtained can be attributed to the suggested mechanisms of the formaldehyde reaction with amino acid chain on the protein molecule (Barry, 1976). Thus, in the case of fish silage, the action of the formaldehyde was two-fold: firstly to inhibit proteolysis of fish protein during autolysis (as indicated by the increased proportion of true protein in the formalin treated silages (see Table 21), and secondly to inhibit proteolysis of this true protein in the rumen.

The levels of formadehyde application used in this work are well within the range of those reported by various workers using different proteins (Ferguson, et al., 1967; Offer, et al., 1971; Peter, et al., 1971 and Schmidt, et al., 1973). Differences in response may be attributed to differences in type of protein, in vitro assay system or in methods used to treat the protein with formaldehyde.

Comparison of feedstuff proteins

The results show that white fish meal protein is able to survive rumen degradation without great degradation as indicated by the small amount of ammonia-N produced. Formalin treated fish silage was more resistant to degradation than the untreated control, casein or urea. The high rate of ammonia-N production associated with urea is in a good agreement with that reported by Chalupa (1968) and Stiles, et al. (1970).

The results also suggest that fish silage, particularly when formalin treated, should be a better protein source for ruminant animals, than urea, casein and untreated fish silage. When comparing formalin treated fish silage with white fish meal it should be noted that the effective degradability in vivo depends on both rate of protein degradation and also retention time in the rumen. If liquid passage

rates from the rumen exceed those for insoluble particles, then the actual degradability of fish silage protein may compare more favourably with that for fish meal than would be expected from consideration only of their relative rates of degradation.

The results are in agreement with the result of Johnsen and Ekern, 1982. Compared with herring meal, supplementation of a hay diet with viscera fish silage increased rumen ammonia-N concentration and molar per cent of iso-butyric and iso-valeric acids, indicating elevated microbial degradation of dietary protein. A high level of formaldehyde (12.6 g per 100 g CP) significantly reduced rumen ammonia-N production and reduced molar per cent of iso-butyric and iso-valeric acids. A low level of formaldehyde treatment (3.0 g per 100 g CP) showed no such effect but tended to improve nitrogen balance. They suggested that the reason why high concentrations of formaldehyde are needed to protect fish silage protein against degradation, is because of the high proportion of low molecular weight nitrogen compounds (amino acids and peptides) in fish silage. Furthermore, the low pH of fish silage is unfavourable for the formaldehyde-protein reaction on which protection depends.

The work of Johnsen and Ekern, (1982) differs from the present study in that the formaldehyde was added after autolysis was completed. In this study the aim of formaldehyde addition was to restrict autolysis during the ensiling process. Protein protection in the rumen, whilst advantageous, was not the main aim. Use of formaldehyde after autolysis is not likely to be beneficial since amino acids are not effectively protected from rumen degradation by this method.

CONCLUSIONS

Untreated fish silage protein was rapidly degraded in vitro. Treatment of fish silage protein with formalin (1 - 5 cm^3 kg^{-1} fish waste) led to a linear decrease in rate of ammonia-N production to a level that was lower than that recorded for urea, casein and untreated fish silage. This treatment should improve the utilisation of fish silage protein by ruminant animals, but required testing since the effect on intestinal amino acid availability is uncertain.

C H A P T E R   F O U R

IN VIVO EVALUATION OF FISH SILAGE

SECTION 1

THE EFFECT OF FISH SILAGE AND OTHER PROTEIN SUPPLEMENTS ON THE PERFORMANCE OF YOUNG RUMINANT CALVES

OBJECTIVE

Research has shown that it is cheaper and simpler to convert fish waste into fish silage rather than fish meal. However, the use of fish silage in the diet of the ruminant calf has not been evaluated. The purpose of the following feeding trial is to compare fish silage with fish meal and urea as a protein supplement in the diet of the growing calf and to investigate the effect of formaldehyde treatment on fish silage.

EXPERIMENTAL

Animals

Thirty Friesian castrated male calves, born over a two week period in August 1984, were purchased from 14 different farms for use in the trial. All animals had been weaned and reared conventionally on a proprietary calf diet. At the start of the experiment on 6 November 1984 their mean age was 10-11 weeks and their mean live-weight was 90.5 kg. Thereafter, the animals were uniformly treated and remained on experiment over the approximate live-weight range 90.5-194.5 kg.

Housing and management

At housing, all animals were weighed, new ear tags fitted and penned individually on concrete floors with sawdust bedding. The pens were cleaned and bedded with fresh sawdust once per week whilst the calves were being weighed. Additional sawdust was provided daily.

Fresh feed and water were offered each day using plastic bins and water buckets fitted to the door of each pen. The animals were weighed once per week prior to feeding.

Production of fish silage

Material

1. Fish waste - fresh whiting, both whole fish and viscera, mixed with cod. The waste was obtained from a commercial processing plant in AYR within 24 hr of the fish being landed.

2. Electrical meat mincer*[1].

3. Additives - a. Add-F*[2] (85% formic acid).
   b. Silaform*[2] (55% formic acid + 26% formaldehyde)

4. 200 litresplastic drums.

5. Small cement mixer.

Method

The fish waste was collected daily as soon after landing as possible. The waste was minced using an electrical mincer fitted with a screen of 10 mm diameter hole size. Immediately after mincing, the fish mince was transferred into the cement mixer and mixed with either 25 kg t^{-1} formic acid (added as 29.4 kg t^{-1} Add-F) or a mixture of 25 kg t^{-1} formic acid + 5 kg t^{-1} formalin (added as 16.9 kg t^{-1} Add-F + 19.2 kg t^{-1} silaform). The fish waste was thoroughly mixed with the additives using the cement mixer. The prepared products (untreated fish silage and formalin treated fish silage) were stored in plastic drums (with lids) as it is corrosive to metal. In each case the pH was initially below 4 to prevent microbial action. After the initial mixing the silage process started naturally, but occasional stirring and heating up to 25°C for 3 days helped to speed up liquifaction as ambient temperatures were low (less than 5°C). The untreated fish silage took about 2-3 days to liquefy, while in the case of formalin treated fish silage it took 6-10 days.

After liquifaction the silages were allowed to stand in the drums for one week before use in order to kill any pathogens present.

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*[1] The Hobart Manufacturing Company Limited, Hobart Corner, New South Gate, London, NI1 1QW.

*[2] BP Nutrition U.K.

During the calf feeding trial, and sheep digestibility trial the fish silages were stored under aerobic conditions at 10°-15°C inside the feed store. Composition of both silages is shown in Table 44.

Experimental diets

A standard basal pelleted diet was supplemented with one of the following supplements:

Supplement	Code
Urea	U
Untreated fish silage	CS
Formalin treated fish silage	FS
Untreated fish silage + fish meal	CSFM
Fish meal	FM

Both urea and fish meal were incorporated into the diet before pelleting, whilst the fish silage was mixed with the diet immediately before feeding. All diets were designed to supply equal energy and crude protein. The composition of the diets is given in Table 45 . Diets FM and U provided the respective positive and negative controls for the fish silage.

Experimental design

The 30 calves were grouped into six blocks of five in the order of reaching 90.5kg live-weight and were randomly assigned within blocks to the five treatments using a randomised block design. The design of the trial is shown in Figure 24 .

Feeding routine

For an acclimatisation period of 1 week after penning, the calves were fed 1.0 kg d^{-1} of proprietory calf feed, in addition to their specific diet . During the next four days the quantity of the proprietory calf feed was reduced and for the last two days of the week the only feed used was the experimental diet . Thereafter, each animal received the same diet for a 15 week period. The diets were given on an _ad libitum_ basis once a day at 9.00 a.m. in quantities adjusted to be 10% in excess of the expected daily consumption (based on the intake for the previous week).

TABLE 44

Composition of fish silage

Composition	Treatment	
	Untreated fish silage	Formalin treated fish silage
Dry matter ($g\ kg^{-1}$)	241.0	250.0
Crude protein ($g\ kg^{-1}$ DM)	578.0	603.0
Oil ($g\ kg^{-1}$ DM)	235.0	249.0
True protein ($g\ kg^{-1}$ CP)	203.0	412.0
Ammonia-N ($g\ kg^{-1}$ CP)	4.0	2.0
Gross energy ($MJ\ kg^{-1}$ DM)	21.2	23.0
pH	3.8	3.9
Total ash ($g\ kg^{-1}$ DM)	124.0	140.0
Ca ($g\ kg^{-1}$ DM)	30.4	37.1
P ($g\ kg^{-1}$ DM)	19.3	23.6
K ($g\ kg^{-1}$ DM)	12.9	12.2
Na ($g\ kg^{-1}$ DM)	7.1	6.1
Mg ($g\ kg^{-1}$ DM)	1.6	1.6

TABLE 45

Composition of the experimental diets (g kg^{-1} DM)

Composition	Diet				
	CS	FS	CSFM	U	FM
Barley	545	543	558	648	570
Barley straw	234	233	227	201	215
Molasses	88	88	88	86	86
Fish meal	0	0	43	0	86
Fish silage	89	89	39	0	0
Urea	0	0	0	18	0
Na_2SO_4	0	0	0	4	0
Minerals	47	47	45	45	45
<u>Nutritive value</u>					
Dry matter (g kg^{-1})	697	699	781	847	849
Crude protein (g kg^{-1} DM)	127	128	129	134	134
Degradability (%)	79	77	74	88	69*
UDP (g kg^{-1} DM)	27	29	33	16	41
RDP (g kg^{-1} DM)	100	99	96	118	93
ME[+] - in vivo (MJ kg^{-1} DM)	11.3	11.2	11.3	11.1	10.7

*The rumen degradability of the protein in the dry feed was measured using the dacron bag method in sheep (see Appendix 8) adjusted for k = 0.05. For fish silages degradability values of 85 and 65 were assumed for the untreated and formalin treated fish silages respectively.

[+]Predicted from measured DE using ME = 0.81 x DE

Figure 24 : Pen distribution showing randomised design

FM 23	FS 24	CSFM 25	CS 26	U 27	FM 28	CSFM 29	FS 30
	CS 22	U 21	U 20	CS 19	CSFM 18	FS 17	FM 16

DOOR

	9 FS	10 CSFM	11 U	12 CS	13 CSFM	14 FM	15 FS
FM 8	U 7	CS 6	U 5	FS 4	FM 3	CSFM 2	CS 1

Fish silage was fed at approximately 25% of the diet on fresh basis for diets CS and FS and at 12.5% for diet CSFM. This allowance was adjusted weekly according to the silage dry matter and the amount of dry diet to be fed. Refusals were weighed every morning and samples retained for dry matter determination.

The daily quantities of each ration for a complete week were weighed into polythene bags, sealed and stored until required for feeding. The required quantities of the fish silage supplements were weighed and stored in plastic containers at room temperature. Fish silage was mixed by hand with the dry part of the ration prior to feeding. To avoid variation in the composition of fish silage during the course of the trial small quantities of fish silage were taken from each 200 litres drum (after stirring) and were mixed together before weighing for feeding.

Sampling and analysis of feed and feed residues

During the weekly weighing of the rations representative feed samples were removed for dry matter analysis. Feed refusals were sub-sampled daily and their dry matter content measured on a weekly composite sample. In addition during the trial, a series of random sub-samples of dry feed and fish silage were taken for chemical analysis. Average analyses are shown in Tables 44 and 45.

Calculation of dry matter intakes

The daily dry matter intakes were calculated from the weights offered and refused. As the fish silage supplement was mixed with three of the diets (CS, FS and CSFM) the dry matter of the fish silage was added to the basal diet dry matter intake to give the total dry matter intake per head per day.

Statistical analysis

The data were analysed as a randomised block design. Total dry matter intakes, daily live weight gains and feed conversion ratios were subjected to the analysis of variance technique to determine statistically significant differences between treatments using the GENSTAT Program suite. Live weight gains for each calf were calculated by regression for weeks 0-8, 9-15 and 0-15.

In a subsequent analysis (see Discussion) of the data the experiment was divided into five 3-week periods and the live weight gains were taken as the average of the differences between the live weights for the first 2 weeks and the second 2 weeks of each 3-week period.

RESULTS

Chemical analysis

Samples of both silages (untreated and formalin treated silages) were analysed before the feeding trial. The results (see Table 44) were used to formulate the diets for the trial. The general composition of both silages was similar. Dry matter content differed slightly due to the inherent lack of homogenity in the raw material. A similar trend was also noticed in the crude protein, oil and energy contents. The breakdown of protein in the formalin treated fish silage was less than for untreated fish silage due to partial preservation by the formalin added. Agreement between the values for ash was not good, suggesting either poor mixing or sampling. The low values for pH and ammonia-N indicate that both silages were well preserved. However, the untreated fish silage developed a slight pungent odour during the later stage of storage. Neither fish silage required neutralisation prior to animal feeding. Representative samples of the oil fraction were taken from both fish silages after processing and the content of long chain-fatty acids determined. The results are given in Table 46. There were no large nor consistent differences in fatty acids composition between the two silages. The results are compared with those obtained from cod liver oil as a standard. Table 46 also shows that levels of unsaturated acids in both fish silages were extremely high (65%), which suggests that extensive oil decomposition seems likely to occur during liquefaction and storage.

The composition and estimated nutritive values were calculated for each diet (see Table 45).

Intake and animal performance

During the period of the trial there was some incidence of ring worm (see Appendix 9), but intake and performance were not affected. The fish silage diets were accepted by animals from 10 weeks of age.

TABLE 46

Proportion of long chain fatty acids in the extracted oil from fish silage

Fatty acid	Fatty acid composition (g/100 g fatty acids)		
	Untreated fish silage	Formalin treated fish silage	Cod liver oil (standard)
C14:0	4.7	4.9	5.0
C14:1	-	-	-
C16:0	16.7	16.4	10.6
C16:1	6.6	6.6	11.8
C18:0	5.3	5.1	3.2
C18:1	19.0	18.7	23.2
C18:2	2.3	2.4	2.8
C20:1	6.6	7.2	12.4
C20:4	3.3	3.3	2.5
C20:5	10.8	11.0	6.9
C22:1	6.5	7.0	7.0
C22:5	1.1	1.0	2.5
C22:6	14.7	14.5	9.5
Minor. components	2.4	1.9	1.7
Sum	100.0	100.0	100.0
% Saturates	26.7	26.4	18.8
% Monounsaturates	38.7	39.5	48.3
% Polyunsaturates	32.2	32.2	23.4

During the later stages the calves on diets CS and FS were offered up to 2 kg d^{-1} fish silage (fresh matter) and some palatability problems occurred.

The animals averaged 90.5kg live weight at the beginning of the trial. Figure 25 shows the changes in mean group live weights of the animals on the 5 diets. The numerical values for each diet can be found in Appendix 10. Figure 25 shows that the animals on diet CS gained live weight at a lower rate than those fed diets FS, CSFM, U and FM. The animals on diet FM achieved the highest rate of gain.

Figure 26 shows the mean group total dry matter intakes of the animals. Calves on diet CS consumed less feed dry matter than animals on diet FS, which in turn consumed less than those on diet CSFM. The animals on diets U and FM had the highest and most constant intakes over the whole period of the trial. The intake and performance data for the individual animals on the different diets can be found in Appendices 11,12 and 13.

__Period 0-8 weeks__

Table 47 shows the mean total dry matter intakes of the animals on the different diets. Intake was least on diet CS (159 kg DM), with the animals on diets FS, CSFM, U and FM consuming 8, 14, 30 and 25 kg DM more respectively. The intake of the animals on diet CS was similar to that for diet FS ($P < 0.05$). The total dry matter consumption for diet U was significantly higher than for diets CS, FS and CSFM ($P < 0.05$) but there was no significant ($P < 0.05$) difference between diets U and FM.

The animals fed diet CS gained 0.05 kg d^{-1} (7.2%), 0.22 kg d^{-1} (31.9%), 0.21 kg d^{-1} (30.4%) and 0.30 kg d^{-1} (43.5%) less weight than those on diets FS, CSFM, U and FM respectively. There were no significant ($P < 0.05$) differences between diets CS and FS or diets U and FM ($P < 0.05$). The weight gain of the animals receiving diet CSFM nearly matched that for those receiving the diet FM and U. (see Table 47).

The feed conversion ratios during this period showed that the animals on diet CS were least efficient as they consumed most feed per unit of weight gain. The observed differences were not significant

FIGURE 25: ANIMAL LIVE WEIGHTS OVER THE WHOLE TRIAL PERIOD.
(MEAN LIVE WEIGHT OF EACH GROUP)

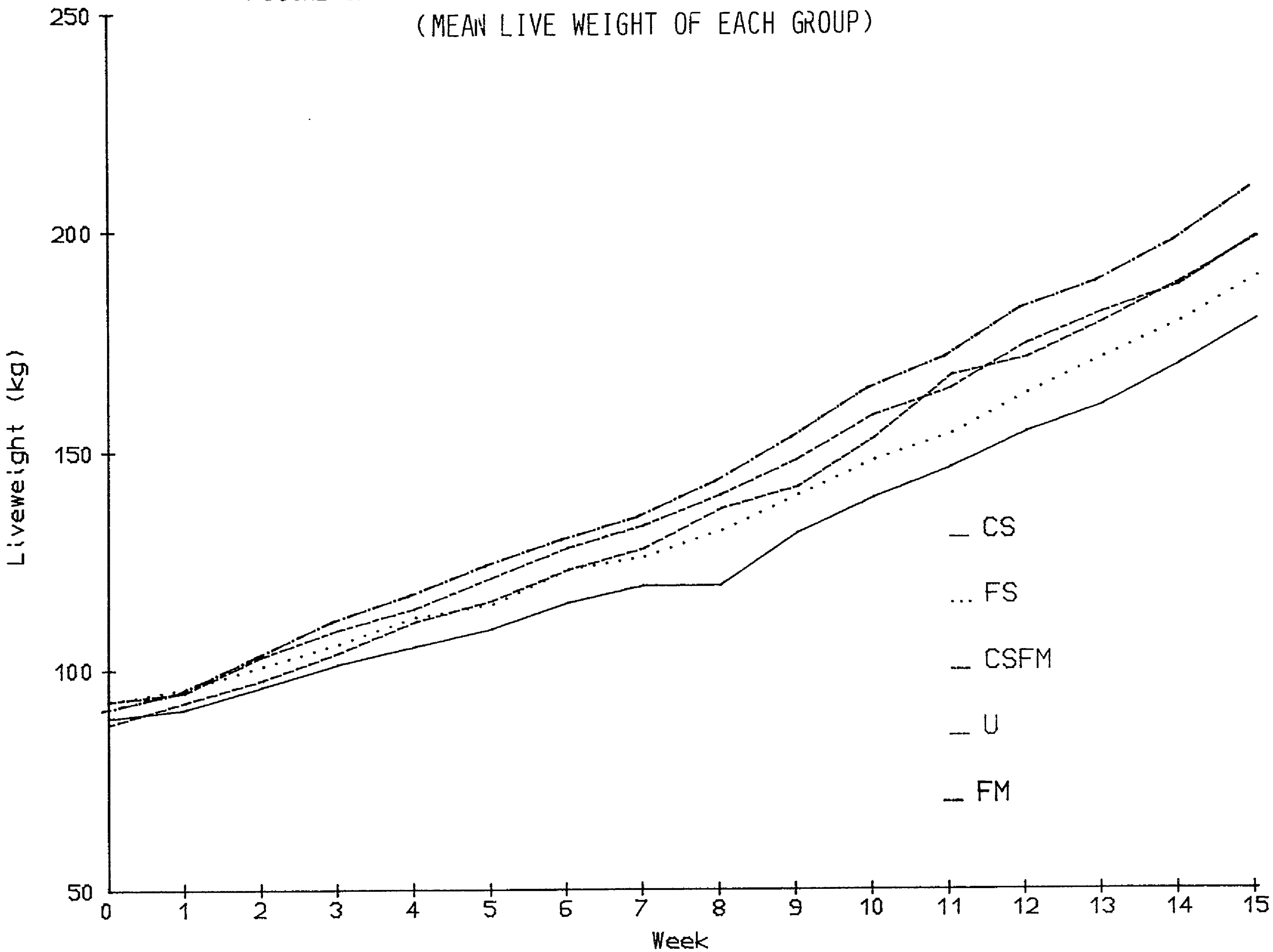
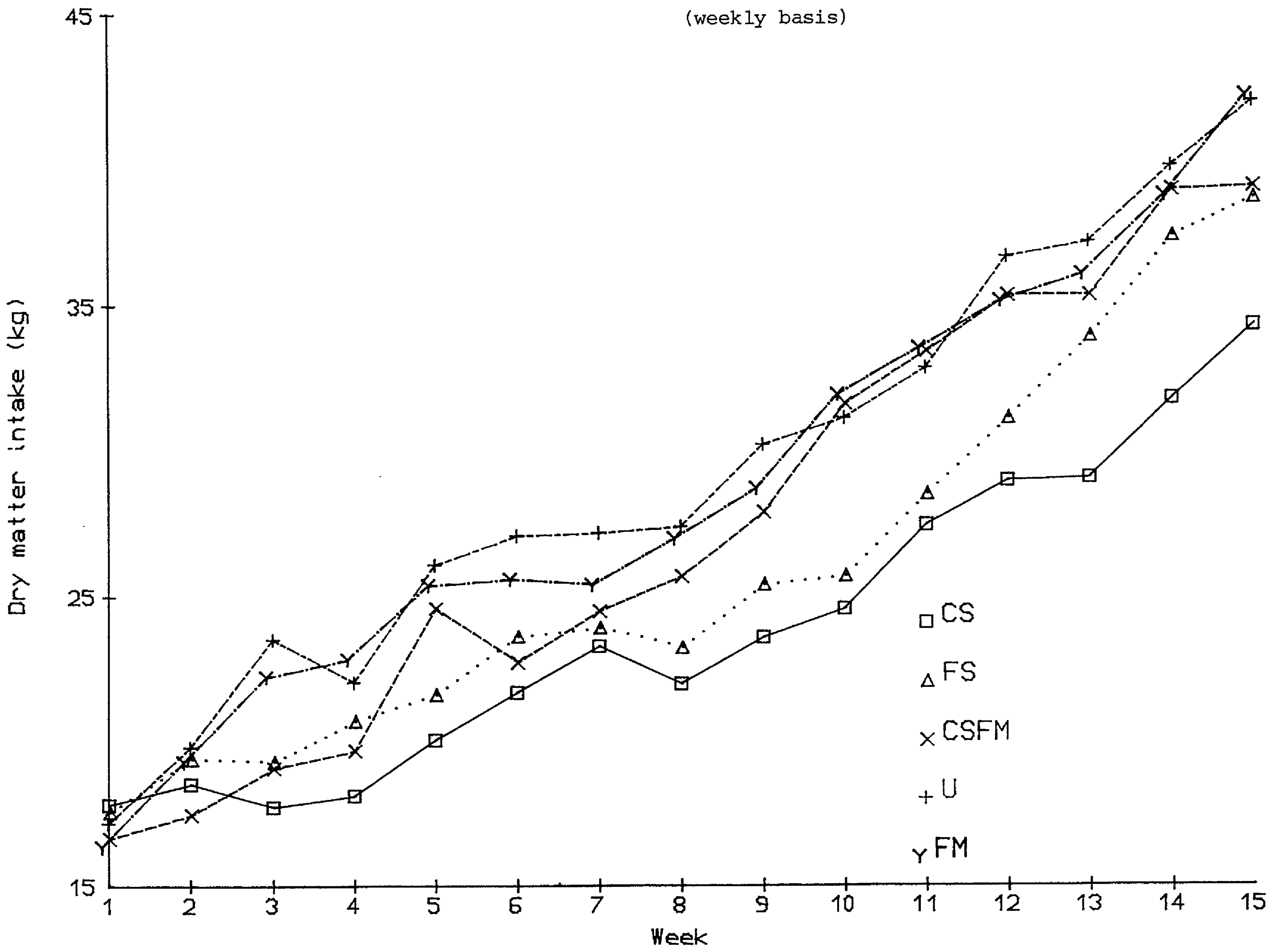


FIGURE 26: MEAN GROUP TOTAL DRY MATTER INTAKES OVER THE WHOLE TRIAL PERIOD.

(weekly basis)



T A B L E 47

Mean intakes and performance of calves*

Period 0-8 weeks[+]

Objective	Diet				
	CS	FS	CSMF	U	FM
Total dry matter intake (kg)	159^{a}	167^{ac}	173^{bc}	189^{d}	184^{bd}
Daily dry matter intake ($kg\ d^{-1}$)	2.84	2.98	3.09	3.38	3.29
Live weight gain ($kg\ d^{-1}$)	0.69^{a}	0.74^{a}	0.91^{b}	0.90^{b}	0.99^{b}
Feed conversion ratio ($kg\ DMI\ kg^{-1}\ LWG$)	4.83^{a}	4.72^{a}	3.96^{b}	4.40^{a}	3.88^{b}

*Means not sharing common subscripts differ significantly ($P < 0.05$)

[+]Intakes and performance of each animal are listed in Apprendix 11.

between diets CS and FS. Animals on diet FM were significantly ($p < 0.05$) more efficient than animals on diet U. Again, animals on diet CSFM were as efficient as animals on diet FM.

Period 9-15 weeks

Table 48 shows the mean total dry matter intakes during this period. The animals on diet CS consumed least (201 kg DM), with the animals on diets FS, CSFM, U and FM consuming 17, 43, 47 and 45 kg more DM respectively. Animals on diet FS consumed 8.5% more DM than those on diet CS. These differences were significant ($P < 0.05$). Compared with both fish silage diets (diets CS and FS) diet DM gave significantly ($P < 0.05$) higher intakes. The same was true for diet CSFM.

The animals fed diet CS gained 0.05 kg d^{-1} (4.5%), 0.19 kg d^{-1} (17.0%), 0.05 kg d^{-1} (4.5%) and 0.16 kg d^{-1} (14.4%) less weight than those on diets FS, CSFM, U and FM respectively. There were no significant differences ($P < 0.05$) between diets CS and FS or between diets CSFM and FM. Daily weight gain was significantly lower for diet U than for diets CSFM and FM ($P < 0.05$).

Diet U gave the lowest feed conversion ratio for this period ($P < 0.05$), but there were no other significant differences among the other treatments.

Period 0-15 weeks

Table 49 shows that during the entire experimental period animals fed the high level of fish silage consumed significantly ($P < 0.05$) less feed than those fed the other diets. The lowest intake was for diet CS which was 25, 55, 76 and 68 kg DM less than the animals on diets FS, CSFM, U and FM respectively. Formaldehyde treatment of the fish silage increased intake over the 15 weeks by 7% but the increase was not significant at $P < 0.05$ (FS compared with CS). The differences in intake between the other diets (CSFM, U and FM) were not significant ($P > 0.05$).

Animals fed the high level of fish silage (CS and FS) gained significantly ($P < 0.05$) less weight than those fed the other diets. The animals fed diet CS had the lowest daily weight gain (0.87 kg d^{-1}) which was 0.05 kg d^{-1} (5.7%), 0.21 kg $^{-1}$ (24.0%), 0.17 kg d^{-1}

T A B L E 48

Mean intakes and performance of calves*

Period 9-15 weeks[+]

Objective	Diet				
	CS	FS	CSFM	U	FM
Total dry matter intake (kg)	201[a]	218[b]	244[c]	248[c]	246[c]
Daily dry matter intake (kg d^{-1})	4.10	4.45	4.98	5.06	5.02
Live weight gain (kg d^{-1})	1.11[a]	1.16[ab]	1.30[c]	1.16[ab]	1.27[bc]
Feed conversion ratio (kg DM I kg^{-1} LWG)	4.33[a]	4.51[a]	4.37[a]	5.17[b]	4.57[a]

*Means not sharing common subscripts differ significantly ($P < 0.05$)

[+]Intakes and performance of each animal are listed in Appendix 12.

TABLE 49

Mean intakes and performance of calves*

Period 0-15 weeks[+]

Objective	Diet				
	CS	FS	CSFM	U	FM
Total dry matter intake (kg)	361[a]	386[a]	416[b]	437[b]	429[b]
Daily dry matter intake ($kg\ d^{-1}$)	3.44	3.68	3.96	4.16	4.09
Live weight gain ($kg\ d^{-1}$)	0.87[a]	0.92[a]	1.08[bc]	1.04[b]	1.13[c]
Feed conversion ratio (kg DM I kg^{-1} LWG)	4.27[a]	4.37[a]	4.01[b]	4.28[a]	3.87[b]

*Means not sharing common subscripts differ significantly ($P < 0.05$)

[+]Intakes and performance of each animal are listed in Appendix 13.

(19.5%) and 0.26 kg d^{-1} (29.9%) less than those fed diets FS, CSFM, U and FM respectively. Again, there was no significant ($P < 0.05$) difference between diets CS and FS ($P < 0.05$). Animals which received diet FM gained significantly ($P < 0.05$) faster than those fed diet U. The overall daily gain of the animals on diet CSFM was similar to that for diets U and FM. Feed conversion efficiency over the whole 15 weeks of the trial was similar for diets CS, FS and U ($P < 0.05$). Diets containing fish meal (CSFM and FM) produced the most efficient conversions which were significantly ($P < 0.05$) lower than for the other treatments.

Analysis of variance for the intake and performance data can be found in appendices 14, 15 and 16.

DISCUSSION

Choice of experimental diet

A major problem of long term animal trials is the difficulty in maintaining a constant composition of the experimental diets. Variation in the nutritive value of forages, both within and between seasons, often makes analysis of the trial more difficult. Thus in the present study, a sufficient batch of the complete diet in a pelleted form was manufactured for use in the calf feeding trial, sheep digestibility trial and passage rate trial to minimise variability in dietary composition. The diet was based on 40% coarsely ground barley straw and 60% ground barley. Molasses (9%) was used to improve the physical form of the pellets. The diet also contains about 5% mineral/vitamin supplements.

Previous investigation of the inclusion of ground straw in concentrate pellets for early weaned calves showed significant advantages in performance over a system in which concentrates and roughage were fed separately. Furthermore, a pelleted complete diet seemed to facilitate a rumen fermentation pattern which promoted rumen papillary growth (Thomas and Hinks, 1982).

There were only small differences in crude protein and energy values

among the five experimental diets (see Table 45) which did not invalidate the trial. The differences in protein and oil content between untreated and formalin treated fish silage (see Table 44) were probably due mainly to variation in raw material. Fish silage was made over nine days and there was noticeable variation in the proportion of different fish species present in the waste. Attempts to ensure that similar material was used to make both silages was made difficult by the fact that only a small amount could be made each day.

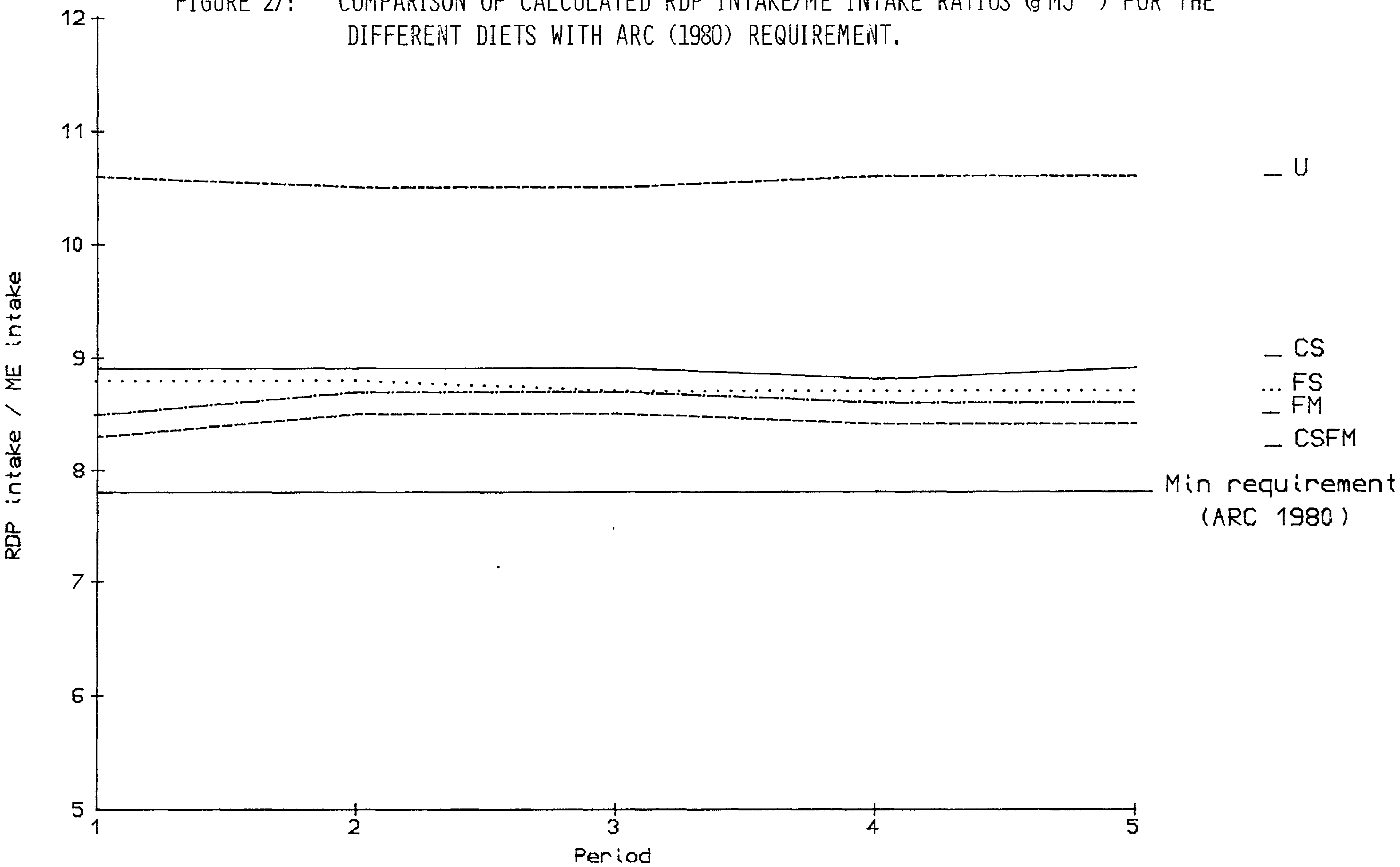
Effect of protein supplements on intake and performance

The results of this trial indicate that fish silage (both untreated and formalin treated fish silage) was inferior to white fish meal or urea in promoting intake and live-weight gain in young ruminant calves.

During the initial period of the trial (0-8 weeks) the animals fed the diets containing the high level of fish silage (diets CS and FS) performed significantly less well than the animals on the other three diets. The superior performance of animals fed the fish meal over those fed fish silage can be explained firstly by the differences in feed intake, since the voluntary consumption of feed dry matter was higher for the animals which received the fish meal supplements. Secondly, the fish meal provided the greatest supply of UDP of high biological value. (All the diets provided sufficient RDP according to ARC (1980), see Figure 27). A supply of supplementary UDP has been shown to be necessary for maximum calf growth up to four months of age (Waterhouse *et al*., 1983; Bax and Offer, 1983; Hughes and Chalmers, 1983 and Smith *et al*., 1982).

Both fish silages were inferior to urea during this initial period. The similarity of live weight gain for diets U and FM was unexpected, since other workers (Kay *et al*., 1967; Stobo *et al*., 1967c; Waterhouse *et al*., 1983 and Bax and Offer, 1983) found urea to give inferior performance at this stage of growth. The high live weight gain for diet U may be attributed to the higher intake on this diet, since the feed conversion efficiency was lower than for diets CSFM or FM. This may have been caused by the slightly higher barley and lower straw content and also to the absence of 'fish taste' of the

FIGURE 27: COMPARISON OF CALCULATED RDP INTAKE/ME INTAKE RATIOS ($g\,MJ^{-1}$) FOR THE DIFFERENT DIETS WITH ARC (1980) REQUIREMENT.



urea diet. There were factors in the trial which would favour efficient use of urea: the early development of the rumen and its microbial population which follows early weaning; the relatively high proportion of barley and molasses in the ration which would supply readily available energy to the microbes; the fact that the urea was incorporated into a complete diet which was only eaten slowly by the young calf; the supply of supplementary sulphur and other trace elements. Nevertheless, there was evidence that calves fed the urea diet were deficient in UDP. Their feed conversion efficiency was similar to those fed fish silage diets but inferior to those fed fish meal during this period of the trial (see Table 47).

The lower performance of the calves fed the fish silage diets (diets CS and FS) was due partly to their lower dry matter intake. However, the similarity of the feed conversion efficiencies for diets U, CS and FS suggests that calves fed the fish silage were also deficient in UDP.

Although feed consumption, live weight gain and feed conversion efficiency did not differ significantly ($P < 0.05$) between treatments CS and FS, treatement with formaldehyde did cause a small improvement in these parameters (5%, 7.2% and 2% respectively). This may be attributed to the fact that formaldehyde treatment may have increased the amount of UDP available for absorption in the lower gut.

The animals on each of the treatments showed increased growth rate during the latter period of the trial (i.e. 9-15 weeks) over the initial period. The animals fed the diets supplemented with the high level of fish silage (diets CS and FS) showed lower growth rates than those fed the fish meal diets, but were now similar to that obtained with the urea supplemented diet. The feed conversion efficiency of animals on fish silage diets was superior to that obtained with urea. Although feed consumption and live weight gain were higher in the case of fish meal supplemented diets, feed conversion efficiencies for diets CS, FS, CSFM and FM were similar during this final period of the trial. Thus, as the trial progressed intake and performance of the calves fed fish silage appeared to improve relative to those on other diets and particularly to those fed urea. One reason may have been that the calves became less

discriminating with regard to the taste of the fish silage. Also their requirement for UDP would have decreased to a level at which it was met by the fish silage supplement (see later part of Discussion) but not by urea. These effects may have been enhanced by a degree of compensatory growth brought about by their growth being restricted due to the lower intake and a deficiency of UDP during the initial period of the trial. Again, there were no sifnificant differences in performance between calves fed untreated and formalin treated fish silage.

Over the whole period of the trial, significantly reduced intakes and live weight gains were recorded for the calves fed the fish silage diets (diets CS and FS) compared with other diets indicating that animals fed the fish silage diets had still not fully compensated for their earlier slow growth. The overall feed conversion efficiency obtained for the fish silage diets was inferior to that for the fish meal supplemented diets (diets CSFM and FM) but similar to that for urea.

An explanation for the low acceptability of the fish silage (particularly during the early part of the trial) is its high content of oil and the possible production of high levels of free fatty acids and their oxidation products due to rancidity reactions during storage. In many cases the hydrolysis of oil has been associated with toxic effects and lowered animal performance (Barlow and Pike, 1977). The high oil content could have upset the digestion in the rumen, although the sheep digestibility trial (see Section 2 of this Chapter) using the present diets suggests that this was not the case, perhaps because the content of oil in the total dry matter was less than 2.0 %. The high levels of unsaturated fatty acids (65%) in the oil fractions extracted from both fish silages (see Table 46) indicate that the fish oil would be highly susceptible to oxidative rancidity. The slight pungent odour that developed during later stages of storage can also be considered a sign of oil oxidation. Tatterson and Windsor (1974) examined the oil fraction of fish silage and showed that there was a significant increase in free fatty acids and a reduction in iodine value during storage (see Chapter 1, page 101). Gildberg and Raa (1977) also reported high levels of free fatty acids in the separated oil from fish silage. These high levels were attributed firstly, to an initial high level of free fatty acids in

the raw material, and secondly, to rapid oil hydrolysis. The work of Johnsen and Ekern (1982) is also relevant. They examined the effect of infusion of 40 g fish oil into the rumens of sheep fed either hay or hay plus soya meal. Oil infusion drastically reduced feed intake. This reduction in intake is also due to disturbed rumen fermentation as indicated by decreased molar ratio of acetic to propionic acid. The effect on feed intake was greater for all roughage diets than on mixed concentrate/roughage diets due possibly to the greater sensitivity of cellulolytic rumen micro-organisms to changes in rumen environment. Removal of oil from fish silage prior to feeding would improve its intake and nutritional value. Another practical alternative is the addition of anti-oxidant (such as ethoxyquin) to fish silage during production. However, in this study the intention was to use as simple a process as possible so that it might have small-scale localised application. There are other factors which can affect the acceptability of fish silage such as the products of autolysis reaction or the effect of the preservative acid. The palatability problem observed in this trial has been reported by other workers in poultry and pig feeding trials (see Chapter 1, page 108). More work is needed to investigate the factors which affect the palatability of fish silage for the calf.

The results obtained in this trial are in contrast with the results reported by Young and Dunn (1975). They carried out a trial with calves fed indoors a basal diet of hay, straw and bruised oats. The basal diet was supplemented either with protein pellets, or fish silage or fed without supplement. The fish silage was absorbed by the fodder and grain, and the animals accepted the mixture readily. The four month trial indicated that the animals fed the diet supplemented with fish silage produced higher live weight gains than the other two groups. At the end of the summer grazing period that followed the feeding trial, the majority of animals fed fish silage had fattened sufficiently for sale. About 50% of those fed protein pellets had reached the same stage, but the majority of animals from the unsupplemented group required feeding before reaching the fat stage. However, the above workers used about 0.9 kg fish silage to supplement the diet while in the present trial double this amount was used. In addition, they used much older animals (250 kg live weight) compared to the animals used in this investigation (90 kg

live weight). The older calves should be able to utilise the fish silage feed without serious palatability problems, and UDP deficiency would not be a problem.

The comparative responses to fish meal and urea are in agreement with the results reported by Waterhouse et al. (1983) using similarly aged animals, but only when the whole trial is considered. They examined complete diets containing either urea or fish meal as the major protein source. During the period of 11-15 weeks of age there were highly significant ($P < 0.01$) differences in live weight gain (unlike the present study). However, during the period 16-24 weeks of age the differences did not reach statistical significance. Over the whole period of the trial (i.e. 10-33 weeks of age) there were significant ($P < 0.05$) differences, but at a lower level of statistical significance than at 11-15 weeks of age. Other work comparing urea and fish meal but using separate forage and concentrate sources has shown increased weight gain with fish meal in the young calf (Bax and Offer, 1983; Hughes and Chalmers, 1983 and Smith et al., 1982). Increased dry matter intakes with fish meal supplemented diets have also been obtained by Garstang (1981); Righton (1981) and Gill and England (1981).

Hennessy et al. (1981) concluded that the improved growth of steers fed fish meal compared with urea was associated with an increased availability of specific amino acids. It is probable that methionine is the first limiting amino acid for growth in calves (Buttery, 1977). Microbial protein produced from a urea diet is less digestible than that of dietary protein (Chalupa, 1978). It may also be too low in methionine to support adequate live weight gain in growing calves (Salter et al., 1979). Further, the energetic efficiency of microbial protein synthesis in the rumen is inadequate to support the ratio of protein:energy required by rapidly growing young calves (Ørskov, 1976).

Effect of time on trial on responses to protein supplementation

Initial evaluation of the trial suggested that the relative performance of the calves on different treatments may have changed during the later half of the trial. Intakes and live weight gains were, therefore, calculated for 3-week periods to investigate this effect more closely.

Live weight gain was calculated as follows:

$$\text{Live weight gain (kg d}^{-1}) = \frac{(LW_2 - LW_1) + (LW_3 - LW_2)}{2}$$

Where,

LW_1 = live weight in week 1 of the 3-week period

LW_2 = live weight in week 2 of the 3-week period

LW_3 = live weight in week 3 of the 3-week period

The results of this 3-week evaluation were also used to examine the effect of protein supplement on calculated efficiency of utilisation of ME for live weight gain (k_f) and to evaluate the protein status of the treatments using ARC (1980). The mean daily dry matter intakes and live weight gains are shown in Tables 50 and 51. The results obtained in this analysis were similar to the results observed in the previous analysis. The slight differences in mean dry matter intake and live weight gain from that obtained in the previous analysis are mainly due to the fact that, in the previous analysis, data were adjusted for initial live weight as a covariate. Tables 50 and 51 show that there is a time effect as there was a highly significant ($P < 0.01$) interaction between dry matter intake or live weight gain and period (see Appendices 17 & 18). Relative to the other treatments, there were higher dry matter intakes and live weight gains for fish silage supplemented diets during later stages of the trial.

Calculation of efficiency of utilisation of ME for LWG (K_f)

The efficiency of utilisation of ME for gain was calculated to provide a more reliable index of the effect of protein supplementation on efficiency of energy utilisation since feed converstion efficiency is based on dry matter intake. The efficiency of utilisation of ME for gain (k_f) was calculated as follows:

1. The ME values for the different experimental diets were calculated from digestible energy values measured in the sheep *in vivo* digestibility trial (see Section 2 of this Chapter) using the following equation:

$$ME = 0.81 \times DE.$$

TABLE 50

Mean* dry matter intakes (kg d^{-1})

Period+	Diet				
	CS	FS	CSFM	U	FM
1	2.57^{ab}	2.68^{ad}	2.54^{b}	2.88^{c}	2.75^{d}
2	2.84^{a}	3.14^{b}	3.19^{b}	3.58^{c}	3.47^{c}
3	3.26^{a}	3.45^{b}	3.72^{c}	4.03^{d}	3.86^{e}
4	3.86^{a}	4.06^{b}	4.78^{c}	4.79^{c}	4.74^{c}
5	4.52^{a}	5.23^{b}	5.39^{c}	5.64^{d}	5.57^{d}
Overall Mean	3.41^{a}	3.71^{b}	3.93^{c}	4.18^{d}	4.08^{d}

*Means in the same line not sharing common subscripts differ significantly ($P < 0.05$)

+Each period covers 3 weeks.

TABLE 51

Mean* live weight gains (kg d^{-1})

Period	Diet				
	CS	FS	CSFM	U	FM
1	0.55[a]	0.61[ab]	0.77[bd]	0.87[cd]	0.97[c]
2	0.75[a]	0.75[a]	0.87[ab]	0.91[b]	0.92[b]
3	0.90[a]	0.95[a]	1.14[b]	1.14[b]	1.32[c]
4	1.06[a]	1.10[a]	1.30[b]	1.14[ab]	1.27[b]
5	1.33[a]	1.19[ab]	1.27[a]	1.10[b]	1.26[a]
Overall Mean	0.92[a]	0.92[a]	1.07[b]	1.04[b]	1.14[c]

*Means in the same line not sharing common subscripts differ significantly ($P < 0.05$) ·

2. The energy stored in live weight gain for each animal was calculated using the ARC (1980) equations (see Appendix 19) which use the live weight gain and live weight to predict the energy stored.

3. The ME available for live weight gain was calculated from the equation:

ME available for gain = ME intake - ME required for maintenance

ME required for maintenance was calculated from the ARC (1980) equations (see Appendix 19).

4. The efficiency of utilisation of ME for live weight gain is then calculated as follows:

$$k_f = \frac{\text{Energy stored in live weight gain}}{\text{ME available for live weight gain}}$$

Where,

k_f = efficiency of utilisation of ME for gain.

The main weakness of the above calculation is that it assumes that the energy of live weight gain is accurately predicted by the ARC (1980) equations. However, there is evidence that different protein supplements may alter the deposition of protein and fat. Alternatives for measuring k_f value are the use of carcass analysis or colorimetry which are both expensive procedures.

A further parameter (NEAP) was calculated to compare the animal performance with that predicted from ARC (1980) on the basis of the intakes of ME.

$$\text{NEAP} = \frac{\text{NE stored in measured live weight gain}}{\text{NE stored in live weight gain predicted by ARC (1980)}}$$

Where,

NEAP = Net energy - Actual/Predicted.

The mean calculated efficiency of utilisation of ME for gain(k_f) for the animals on the different diets are listed in Table 52 . The data indicate that the ME of diets CS, FS and U was, overall, significantly ($P < 0.05$) less efficiently used than that of diets supplemented with fish meal (diets CSFM and FM). The ME of diet

TABLE 52

Mean* calculated efficiencies of utilisation of ME for live weight gain (k_f)

Period	Diet				
	CS	FS	CSFM	U	FM
1	0.31^{a}	0.35^{b}	0.48^{c}	0.46^{c}	0.62^{d}
2	0.41^{a}	0.37^{a}	0.42^{a}	0.38^{a}	0.45^{a}
3	0.45^{a}	0.47^{a}	0.52^{a}	0.47^{a}	0.69^{b}
4	0.47^{ab}	0.48^{ab}	0.41^{ab}	0.41^{a}	0.53^{b}
5	0.54^{a}	0.40^{bc}	0.42^{bc}	0.34^{b}	0.46^{ac}
Overall Mean	0.43^{a}	0.41^{a}	0.46^{b}	0.41^{a}	0.55^{c}

*Means in the same line not sharing common subscripts differ significantly ($P < 0.05$)

FM was significantly ($P < 0.05$) more efficiently used than that of diet CSFM. It is clear from Table 52 that animals fed fish silage and urea supplemented diets used ME less efficiently during the early stage of the trial than those fed fish meal. This may be attributed to the limited supply of UDP from the fish silage and urea diets compared with the animals requirement at this stage. As the animals grew older there was marked improvement in efficiencies for the fish silage diets possibly due to the UDP supply from these diets meeting the decreasing UDP requirement. Analysis of variance are shown in Appendix 20.

Table 53 shows the mean values for NEAP. There were no significant ($P < 0.05$) differences among animals fed diets CS, FS and U. The animals on diets CSFM and FM produced significantly ($P < 0.05$) higher values over the whole period of the trial than those fed diets CS and FS. The live weight gain for animals fed diet FM (as measured by NEAP) was 20% higher than that predicted by ARC (1980), whilst for urea and fish silage supplemented diets the values were approximately 10% less than predicted. Analysis of variance are shown in Appendix 21.

Evaluation of the protein status of the different diets

The UDP and RDP intakes for the different treatments were calculated from dry matter, crude protein and degradability of each experimental diet (see Table 45). It can be seen from Figure 28 that the UDP intakes (gd^{-1}) for all diets during the early stage of the trial were less than the UDP requirement recommended by the ARC (1980) due to the high UDP requirement for rapidly growing young calves. However, the scale and persistency of the calculated UDP deficiency for the treatments decreased in the following sequence: U, CS, FS, CSFM, FM. Animals fed diet FM were only in small UDP deficit for 1-2 weeks, whilst those fed diet U experienced great deficiency for the first 12 weeks of the trial (see Table 54 and Figure 28). Therefore, the growth responses to protein supplementation are broadly in agreement with that predicted from consideration of dietary UDP status according to ARC (1980). The performance response to the fish meal supplement agrees with other trials (Kirby, 1981; Bax and Offer, 1982, 1983; Waterhouse, 1983).

The differences in live weight gain and k_f between diets are great in the early part of the trial but become less apparent for periods

TABLE 53

Mean* calculated values of NEAP+

Period	Diet				
	CS	FS	CSFM	U	FM
1	0.62[a]	0.71[a]	0.97[b]	0.96[b]	1.31[c]
2	0.83[ac]	0.76[a]	0.87[ac]	0.83[ac]	0.99[bc]
3	0.92[a]	0.98[a]	1.09[a]	1.03[a]	1.50[b]
4	0.99[a]	1.02[ab]	1.02[ab]	0.92[a]	1.20[b]
5	1.16[a]	0.90[bc]	0.96[bc]	0.80[b]	1.07[ac]
Overall Mean	0.90[ab]	0.87[a]	0.98[b]	0.91[ab]	1.21[c]

*Means in the same line not sharing common subscripts differ significantly ($P < 0.05$)

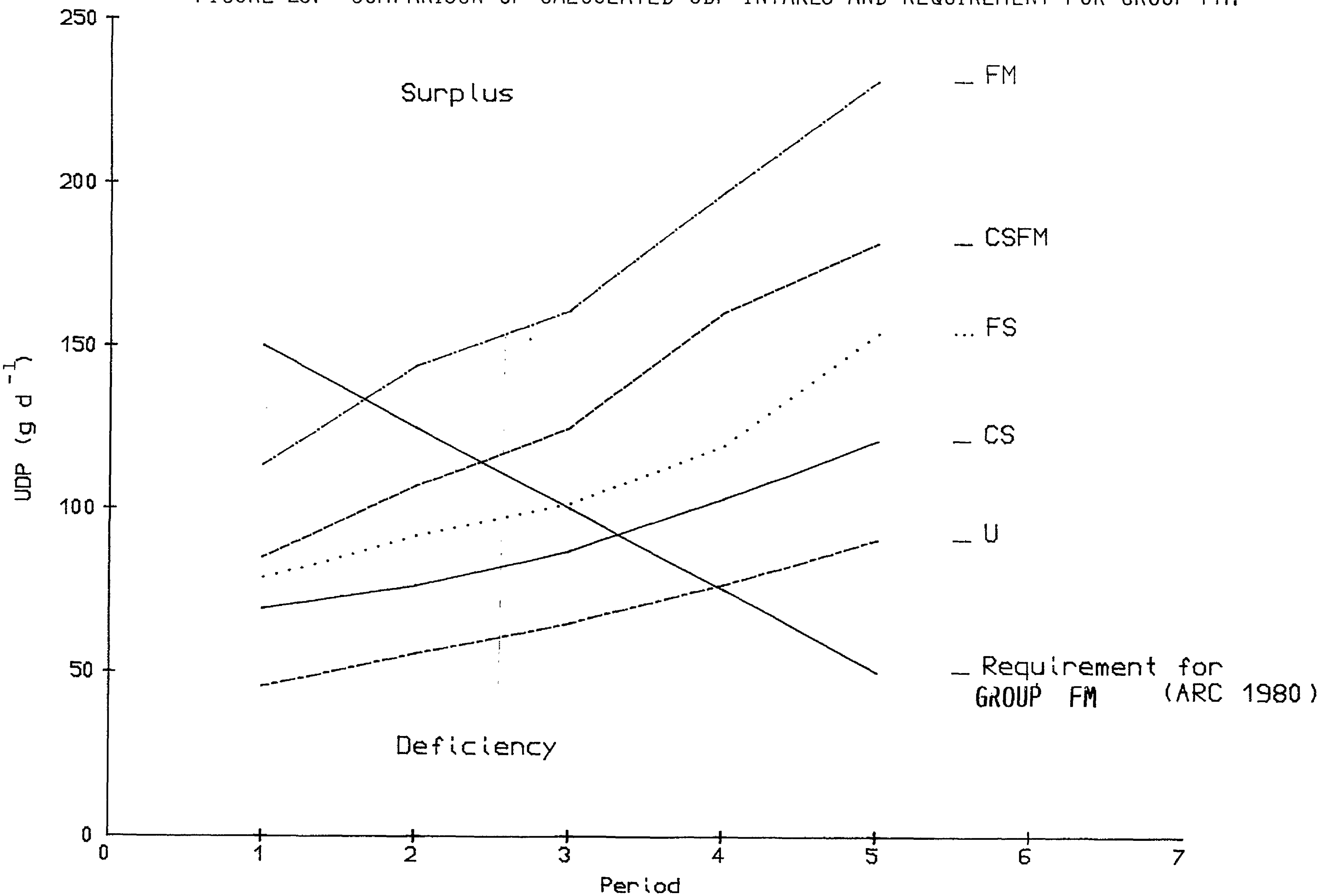
$$+NEAP = \frac{\text{NE stored in measured live weight gain}}{\text{NE stored in live weight gain predicted by ARC, 1980}}$$

TABLE 54

Mean calculated UDP intakes (g d^{-1})

Period	Diet					Requirement for FM group ARC (1980)
	CS	FS	CSFM	U	FM	
1	69	79	85	46	114	157
2	76	92	107	56	144	145
3	87	102	125	65	161	133
4	103	120	160	77	197	126
5	121	154	181	91	231	52

FIGURE 28: COMPARISON OF CALCULATED UDP INTAKES AND REQUIREMENT FOR GROUP FM.



4 and 5 (see Tables 51 and 52) because the increasing UDP supply (due to increased DM intake) parallels the decreasing UDP requirement.

Analysis of the individual calf data reveals that there is low correlation (r = 0.20) between ration UDP and measured live weight gain during the five periods. This is in contrast to the results reported by Bax and Offer (1983), who obtained a higher relationship between UDP intake (gd^{-1}) and live weight gain. However, better relationship (r =0.839) was observed between ration UDP and k_f for the five periods of the trial (see Figure 29). This difference can be attributed to the typical intake responses to urea and fish meal observed in this trial. Figure 27 shows that all the diets supplied adequate RDP for microbial growth. The calculated RDP intake : ME intake ratios ($g\ MJ^{-1}$) for the five diets were found to be above the minimum requirement (7.8) stated by the ARC (1980). The numerical value for each diet is listed in Table 55 .

Some of the reduction in live weight gain for animals on fish silage supplemented diets was due to the decreased supply of protein and energy which resulted from reduced dry matter intake. Also, part of this reduction in gain was, particularly in the early part of the trial, due to lowered efficiency of utilisation of ME for gain probably caused by the inability of the fish silage supplement to meet the potential UDP requirement of the growing calf.

Financial appraisal

The benefits of the fish silage process over fish meal production are the simplicity of silage process, its low capital investment and its energy saving and environmental. Economic studies using poultry and pigs have been undertaken in Sri Lanka (Jayawardena, et al., 1978), in Mexico (Edwards and Disney, 1980) and in the U.K. (Nicholson, 1976). All concluded that fish silage production was a viable alternative to fish meal and other conventional protein supplements (see Chapter 1, page 106). However, there is a lack of experimental data collected under farm conditions in the U.K. to demonstrate that using fish silage as a protein supplement for ruminants is financially worthwhile.

In terms of the cost per unit of protein the fish silage compares favourably with other protein sources such as fish meal. Even when

FIGURE 29: RELATIONSHIP BETWEEN Kf AND RATION UDP (g kg^{-1})

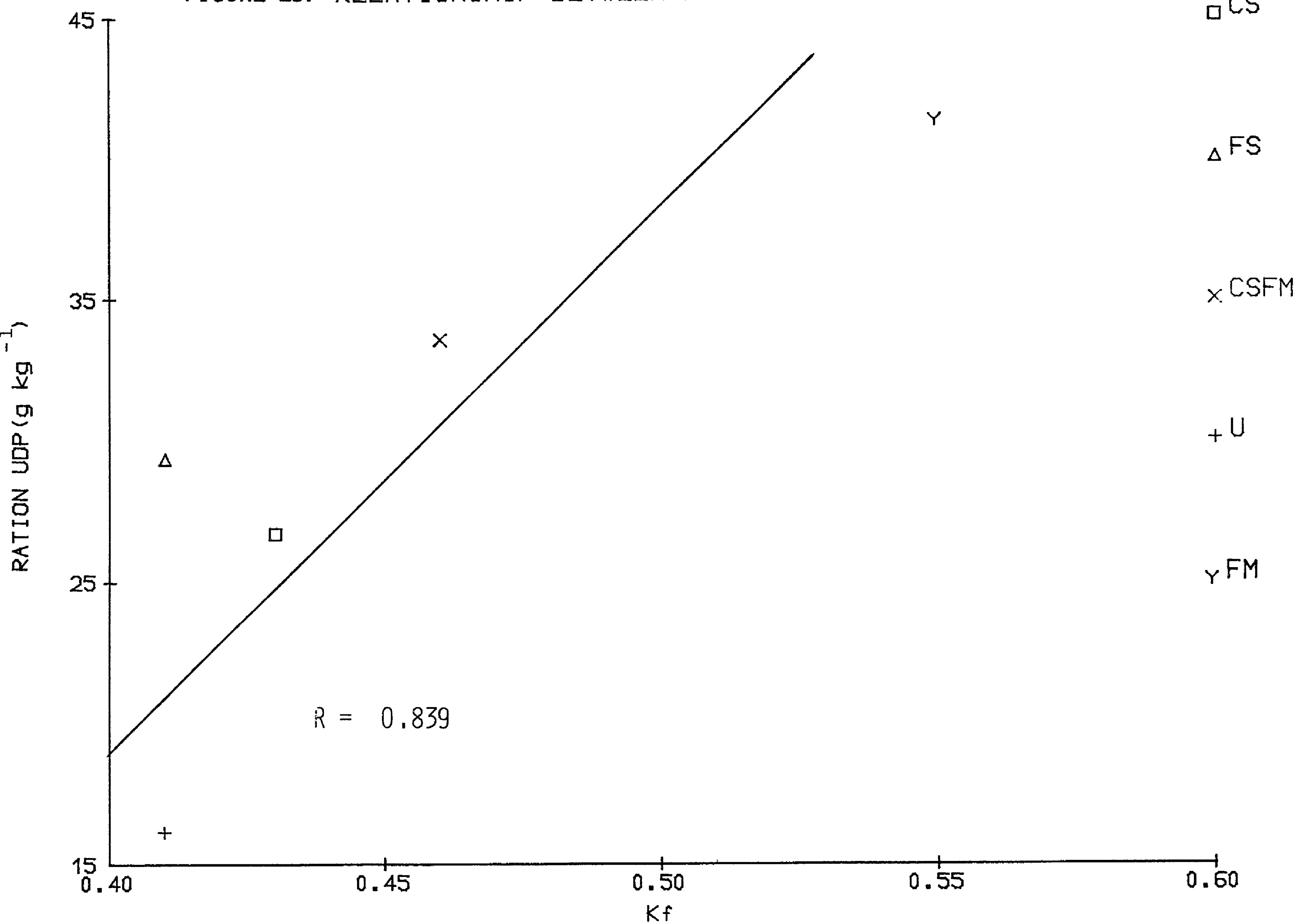


TABLE 55

Mean calculated RDP intake: ME intake ratios for the different diets (g MJ^{-1})

Period	Diet				
	CS	FS	CSFM	U	FM
1	8.9	8.9	8.3	10.6	8.5
2	8.9	8.8	8.5	10.5	8.7
3	8.9	8.7	8.5	10.5	8.7
4	8.8	8.7	8.4	10.6	8.6
5	8.9	8.7	8.4	10.6	8.6
Overall Mean	8.9	8.8	8.4	10.6	8.6

a relatively high cost for preservative acid and fish waste are assumed the costs of production for fish silage are lower than for fish meal. At lower raw material costs, as should be the case if fish waste is used, fish silage is far cheaper than fish meal or soya meal. However, financial appraisal based on the unit cost of protein has limitations and in order to test the economics of fish silage production a wider approach should be adopted. Apart from establishing the cost of fish silage production, the economics of the whole production system must be considered. The type of livestock, feeding system, complementary feeds, fish silage intake, animal performance and total ration cost are all important. The cost of the ration and the value of animal production should be compared with those resulting from alternative feeds.

The physical performance, feed intake data and ration details are given in Table 56 , together with some economic parameters. The appraisal is based only on the data collected during the second half of the trial (i.e. 9-15 weeks). This is because of the conclusion that fish silage is unsuitable for the very young calf because of its poor acceptability.

The total ration cost was greater for the urea and fish meal supplemented diets due to the high intakes achieved and their higher costs per tonne DM. The lower live weight gain for the fish silage diet compensated for this to some extent. However, the gross margin obtained with fish silage (excluding the cost of fish waste) was higher than for the urea and fish meal diets. Thus to match the economic performance of the urea diet the farmer could afford to pay £67 t^{-1} fresh weight for the fish waste. To make the same margin as that observed with fish meal he could, however, afford to pay only £22 t^{-1} for the fish waste. If the cost of the fish waste exceeds £22 t^{-1} this trial shows that fish meal is a better buy for the farmer. If the cost of transporting, mincing and mixing the fish waste is included then this figure is even lower.

The evaluation suggests that fish silage production should not be considered as a large scale alternative to fish meal manufacture. However, where fish silage is produced in small amounts, intermittently or in remote areas its cost to the farmer should be very small. In these circumstances the trial shows that fish silage can be used to financial advantage in beef production.

TABLE 56

Comparison+ of production cost for the second half of the trial (9-15 weeks)

Objective	Diet		
	U	CS	FM
Ration cost ($£t^{-1}$ DM)	119.5	107.3*	130.7
Feed consumed (t/calf)	0.248	0.201	0.246
Total feed cost (£/calf)	29.6	21.6*	32.2
Live weight gain (kg/calf)	56.6	54.0	62.0
Value of live weight gain (£/calf)	64.0	61.0	70.0
Gross margin (£/calf)	34.4	39.4*	37.8

Raw material costs assumed

Barley	113.6 $£t^{-1}$
Straw	23.0 $£t^{-1}$
Fish meal	280.0 $£t^{-1}$
Urea (feed grade)	240.0 $£t^{-1}$
Molasses	80.0 $£t^{-1}$
Mineral mix	300.0 $£t^{-1}$
Add-F	585.0 $£t^{-1}$
Fat stock	113 p kg^{-1} LWG

+This simple comparison does not take into account the cost of processing, labour and transport involved

*Includes cost of Add-F (£14.6 t^{-1} fish waste) but not the cost of fish waste.

CONCLUSIONS

1. Fish silages were produced from white fish waste preserved with either 2.5% (w/w) formic acid, or 2.5% (w/w) formic acid + 0.5% (w/w) formalin on a farm scale. The silages were consumed by calves and appeared stable over a storage period of three months.

2. Over the 15 weeks of the trial, fish silage promoted less intake and LWG than urea or fish meal. However, FCE and calculated k_f values were similar to urea, but significantly less than fish meal.

3. The relative merits of fish silage as a protein supplement improved during the course of the trial. The depression in DMI and LWG compared to the other supplements became less. For the last half of the trial fish silage promoted an FCE and k_f that was superior to urea but less than fish meal.

4. Although the protein supplements had different effects on feed intake, the observed FCE and k_f values during the course of the trial were consistent with an evaluation using the ARC (1980) protein system.

5. Formaldehyde treatment of fish silage had little benefit, although there was a small improvement in feed intake.

6. The depression in feed intake caused by fish silage supplementation may have been related to its high content of unsaturated oil. If high levels of inclusion are used, particularly for young animals, methods of removing or stabilising the oil should be employed.

7. Over the 15 weeks of the trial, urea was inferior to fish meal as a protein supplement for the growing calf in terms of LWG, FCE and calculated k_f value.

8. An appraisal of the latter half of the trial suggested that fish silage may prove an economically viable protein source for beef production, provided the fish waste can be obtained cheaply (less than £22 t^{-1}).

SECTION 2

THE EFFECT OF THE VARIOUS PROTEIN SUPPLEMENTS ON DIET DIGESTIBILITY

OBJECTIVE

The aim of this experiment was to investigate the effect of the various protein supplements used in the calf feeding trial on digestibility measured *in vivo* using sheep.

EXPERIMENTAL

Animals

Five mature Suffolk wether sheep (average live weight 80 kg) each of which had been fistulated and fitted with a permanent rumen cannula were used.

Housing and management

The sheep were individually housed in wooden metabolism crates. Each crate was fitted with a slatted floor and provided with a detachable feed box at the front, together with a removable plastic bucket. Fresh drinking water was provided *ad libitum* throughout the duration of the trial. Live weights of the sheep were recorded prior to crating.

During the trial period, each animal was fitted with a faecal collection harness to which a polythene faeces collection bag was attached. The harness was adjusted by four metal clips and elastic bands. This experimental system allowed accurate measurement of feed intake and faecal output.

Experimental design

The five experimental diets were given according to a 5 x 5 Latin-square design (see Table 57).

Experimental diets

As described previously for the calf feeding trial (see Table 45). The sheep were offered 0.039 kg dry matter per kg metabolic live weight ($W^{0.75}$) per day.

TABLE 57

Experimental design of digestibility trial*

Period \ Sheep No.	86	89	84	90	83
1	CF	CS	U	FM	CSFM
2	CS	CF	CSFM	U	FM
3	U	FM	CF	CSFM	CS
4	FM	CSFM	CS	CF	U
5	CSFM	U	FM	CS	CF

*See page 214 for explanation of diet codes

Feeding routine

An acclimatisation period of seven days was given, during which all animals were fed 1.0 kg d^{-1} of the complete diet FM which contained the high level of fish meal. Each animal then received its specific diet for ten days. Weighing of diets and the feeding procedures used were as described for the calf feeding trial (see page 214). The diets were given twice daily at 8.45 a.m. and 4.45 p.m. The quantities were estimated to provide the maintenance energy requirement of sheep plus 10%. Feed consumption was recorded daily during the experimental periods.

Collection, Sampling and Preparation of Material for Analysis

Feed

During weighing of the dry component of the diet a representative sample was removed, milled and stored in sealed containers at 5°C. Fish silage samples were homogenised using a food mixer and stored in plastic buckets in the freezer at -20°C.

Feed refusals

Any residues remaining from the previous feed were removed immediately prior to the next feed. The residues for the last three days of each period were retained, weighed and stored at 5°C.

Faeces

Faeces were removed from the collection bags at 8.00 a.m. for the first seven days in each ten day period faeces were discarded, but were retained every day for the subsequent three days. The fresh weight of faeces was recorded and the three days collection was stored in a sealed plastic bucket at 5°C. At the end of each three days collection the total faeces was weighed and thoroughly mixed using a food mixer. Two representative samples were taken for drying and subsequent analysis.

Analytical Methods

Dry matter

All feed, feed residues and faeces samples (except fish silage - see page 136) were dried in tiered metal trays for 24 hr at 100°C in a forced draught oven. Duplicate samples, each of 400 g, were used

in all cases. The oven dried samples were then ground to pass a 6.6 mm sieve in a Christie and Norris* mill prior to subsequent analysis.

Organic matter

Duplicate 5 g sub-samples of each ground and dried material were placed in pre-weighed crucibles and then ashed in an electric muffle furnace at 460°C for 17 hr. The crucibles were cooled in a desiccator for 30 min and weighed. The ash was then moisted with distilled water and dried at 100°C for 30-45 min . Ashing was continued at 460°C for a further 5-6 hr to obtain ash free of carbon. The crucibles were then cooled and reweighed and the organic matter content calculated.

RESULTS

The sheep readily accepted both fish silage diets. The mean values obtained for the digestibility of dry matter, organic matter, energy and ME are shown in Table 58 . The values have been calculated from intake and faecal excretion measured over the last three days of a ten day period on each experimental diet. The ME values were calculated from the following equation:

$$\text{ME (MJ kg}^{-1}\text{ DM)} = 0.81 \times \text{DE (MJ kg}^{-1}\text{ DM)} \quad \text{(ARC, 1980)}.$$

Full analysis of variance was carried out on all the data obtained for the five diets (see Appendices 22, 23, 24 and 25. Complete tabulated results are presented in Appendix 26 . There were no significant ($P < 0.05$) differences between diets CS, FS, CSFM, U and FM in digestibility of dry matter, organic matter or energy or ME values. There were small differences between the different periods which reached significance ($P < 0.05$) for DE (MJ kg^{-1} DM) values but not for dry matter, organic matter or energy digestibility values. No significant ($P < 0.05$) differences were found between animals. The ME value of the fish meal diet (diet FM) tended to be lower than for the other four diets, but the difference was not significant ($P < 0.05$).

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*Christie and Norris Ltd., Kings Road, Chelmsford, CM1 1SB, U.K.

TABLE 58

Apparent digestibilities of the experimental diets

Diet	Mean apparent digestibility			ME* MJ kg^{-1} DM
	Dry matter (g kg^{-1})	Organic matter (g kg^{-1})	Energy (KJ MJ^{-1})	
CS	747.60	779.40	761.60	11.34
FS	749.40	781.20	760.00	11.24
CSFM	752.60	788.40	766.60	11.31
U	752.60	781.00	756.80	11.11
FM	743.20	779.80	744.60	10.73
SED ±	15.27	14.55	16.82	0.285
Significance	NS	NS	NS	NS

*Estimated from ME = 0.81 x DE

DISCUSSION

Because of the limited time and resources available the trial was planned for five sheep. It was realised at the outset that results obtained with such a small number of animals are less reliable than these obtained using a large number. The Rowett workers (DAFS, 1975) have reported differences between sheep up to 7% for the digestibility of dry matter. They also observed marked differences in digestibility between one feeding period and another. However, the results available from the present trial indicate non-significant digestibility differences between diets (see Appendices 22, 23, 24 and 25). Forbes et al. (1946) suggested that five sheep per treatment is a sufficient number for most purposes provided that the experimental technique is effective.

In digestion studies using small numbers of animals, the experimental design is extremely important. Designs such as the Latin-square or cross-over are most likely to result in meaningful data since in such designs each animal serves as its own control. With other designs large differences among treatments must occur before the latter become statistically significant.

The ten-days which was adopted for each dietary treatment might be criticised on two counts. Firstly that seven days is not an adequate time interval for the animals or the rumen micro-organisms to become fully adjusted to each diet, and secondly that a three day period is less than optimal for collection of faeces. However, the small differences in digestibility (see Table 58) obtained with sheep on the different diets is evidence that the adaption and collection periods were adequate.

Another problem which is frequently encountered in digestibility experiments is that of ensuring a constant composition for each experimental diet throughout the duration of the trial. The investigation of the Rowett workers (DAFS, 1975), using a complete diet, indicated that composition of the diet varied to an appreciable extent not only between batches, but also within batches of the diet. Because of this finding, many of the differences in digestibility between periods on the same diet, which were observed by these workers, were attributed to the non-homogenous nature of the diet. However, the fact that only negligible variation in chemical

composition was occurred during different periods of the trial suggested that non-homogeneity of the diet was not a serious problem in this trial.

Mineral supplementation (see Table 45) was provided for each diet to avoid deficiencies of both major and trace elements. In the case of the urea diet (diet U) additional sulphur was provided (as Na_2SO_4).

The level of intake achieved throughout the trial was that which would satisfy the maintenance requirement of each animal plus 10% according to ARC (1980).

The results indicate that the replacement of fish meal or urea supplements with either untreated fish silage or formalin treated fish silage did not affect dry matter, organic matter, or energy digestibilities. The high digestibility values of diets supplemented with fish silage indicated that the energy of both fish silages was well digested.

According to the ARC (1980) the basal diet, without additional protein supplement, would have been deficient in RDP (RDP:ME = 5.6 g MJ^{-1}) leading to lowered digestibility. The absence of any significant digestibility differences between the different diets may have occurred because the RDP:ME ratios (g MJ^{-1}) were similar and above 8 g MJ^{-1} for the different diets (see Table 55). This supports the assumption in ARC (1980) that if more than 7.8 g RDP is provided in the rumen per MJ of ME fed then maximum efficiency of microbial growth and activity will be achieved. McAllan and Smith (1983b) (cited in McAllan and Smith, 1983c) showed that there were no differences in efficiencies of microbial protein synthesis between urea and fish meal supplemented diets.

Johnsen and Ekern (1982) compared untreated viscera fish silage, formaldehyde treated fish silage (3 g formaldehyde per 100 g CP treated after autolysis), soya bean meal and urea as protein supplements for sheep fed a basal diet containing grass silage plus barley/oat mixture. Supplementation with fish silage (both treated and untreated) led to a small but significant ($P < 0.05$) increase in digestibility of dietary OM compared to the urea supplement. There was no difference between fish silage and soya bean meal.

This result is similar to the present experiment and the small increase in OM digestibility for fish silage and soya bean meal compared with urea may have been due simply to the additional digestible OM supplied by the supplements.

The observation that fish meal promoted a similar digestibility to urea does not agree with the finding of Bax and Offer (1982) who found that fish meal supplementation led to improved digestibility. The reasons for this difference are unknown, but could be related to the rates of digestion of the protein and energy component of the differenet feeds. However, the digestibility responses to fish meal have proved unreliable (Bax and Offer, 1983).

CONCLUSIONS

a. The level of formalin used to treat fish silage protein (5 cm^3 kg^{-1} fish waste) did not reduce the diet digestibility in sheep.

b. Diets supplemented with fish silage were as well digested as those supplemented with fish meal or urea.

c. There were no differences in digestibility between diets supplemented with either fish meal or urea.

d. Differences in performance in the calf feeding trial could not be attributed to differences in diet digestibility.

SECTION 3

THE MEASUREMENT OF PASSAGE RATE

OBJECTIVE

The aim of the experiment described below was to investigate the passage rate of soluble and small particle fractions from the rumen of the calf.

EXPERIMENTAL

Animals

Six of the castrated Friesian calves used in the feeding trial were used in this experiment. The calves weighed an average 164 kg live weight and were 23 weeks old at the start of the experiment.

Housing and management

The animals were housed and managed as described for the feeding trial (see page 212) with the exception that fresh sawdust bedding was necessary every 2 hr during faeces collection to enable faecal sampling. Uneaten feed was mixed and sampled daily and dried to calculate animal dry matter intake.

Diet

During the experimental period the calves were offered the ration CS (they formed a group in the main calf feeding trial). The ration comprised a basal dry diet supplemented with 25% untreated fish silage (see Table 45 for details).

Dosing procedure and experimental design

The six calves were divided into two groups of three. A cross-over design using two treatment periods was used. In each period 250 cm^3 of Cr-EDTA complex was introduced for each animal in one group, whilst at the same time the other group received a solution containing 50 g of Cr-treated fish meal (see Chapter 2 for markers preparation). The experimental design is shown in Table 59. The markers were introduced 1 hr after feeding. One hour was allowed for

TABLE 59

Experiment design*

Period	Animal No.					
	Group 1			Group 2		
	1	19	22	12	26	6
1	Cr-EDTA			Cr-treated fish meal		
2	Cr-treated fish meal			Cr-EDTA		

*Cr-EDTA and Cr-treated fish meal were used as markers for the soluble and small particles fractions of the digesta respectively.

eating, and mid-point in this hour was taken as the time of feeding.

The markers were administered orally using a long-necked plastic bottle. Care was taken to avoid the loss of part of the dose by splashing caused by sudden movement of the animal. No ill effect of administration of Cr-EDTA or Cr-treated fish meal to calves was observed.

Faeces collection

Faecal collection were made during each of the two experimental periods. The two periods were separated by 7 days, when no collections were made, to make sure that the faeces were free of any residual Cr before the second dose. Faecal collection commenced 7 hr after dosing for the Cr-EDTA group and 11 hr for the Cr-treated fish meal. Subsequent collections for both groups were made at 2-3 hr intervals for the next 36 hr. The faeces were scraped from the sawdust floor under each calf, placed in a plastic bucket and well homogenised by hand. Each faeces sample comprised three or four small portions taken from different parts of the homogenised faeces. The samples were collected in aluminium foil trays. All faeces were removed from each calf pen and fresh sawdust was spread throughout.

Statistical analysis

The experiment was analysed as a cross-over design. Rumen passage rate were determined from the concentration of Cr in the faeces as described by Grovum and Williams (1977) using a computer program for the linear regressions. All data were subjected to the analysis of variance.

RESULTS

In calculating and measuring passage rate from the rumen, the method of Grovum and Williams (1973b) was used to fit the two compartment model described by the following equation to the faecal excretion data:

$$Y = Ae^{-k_1(T-TT)} - Ae^{-k_2(T-TT)} \qquad T > TT$$

$$Y = 0 \qquad T \leqslant TT$$

Where Y is the concentration of marker in the faeces;

k_1 represents passage rate of marker from the reticulo-rumen;

T is the time elapsed since introducing the marker;

TT denotes the time of first appearance of marker in the faeces;

A is biologically undefined;

and k_2 is thought to be the passage rate of marker post ruminally in the caecum and colon (Grovum and Williams. 1973b).

The importance of this approach is that ruminal passage rate can be calculated from the faecal excretion of the marker. A computer program was used in calculating passage rates based on the procedure used by Grovum and Williams (1973b). If the rate of removal of marker from the reticulo-rumen is exponential with time, then the plot of the natural logarithm of faeces Cr concentration should be linear for the excretion curve (after 36 hr in this experiment). The value of the regression coefficient (slope) for this plot is k_1 The data point at which to start this linear regression was selected by choosing the set that gave the highest correlation coefficient. The computer program used made this easy since all k_1 and k_2 values were calculated as individual points were sequentially removed from the start of the plot.

Table 60 shows the values of k_1 and k_2 obtained using Cr-EDTA or Cr-treated fish meal. The values are the data collected over the two periods of the experiment. The overall mean passage rates from the rumen (k_1) for Cr-EDTA and Cr-treated fish meal were 0.0779 hr^{-1} and 0.0524 hr^{-1} respectively. The mean post ruminal passage rates (k_2) were 0.26 hr^{-1} and 0.17 hr^{-1} for Cr-EDTA and Cr-treated fish meal respectively. The values of k_1 and k_2 for Cr-EDTA were higher than that for Cr-treated fish meal by 33% and 35% respectively. These differences were highly significant ($P < 0.01$). The differences between the two markers in passage rates (k_1 and k_2) were almost the same with all animals. The differences between calves and periods were not significant (see Appendix 27). However, small amount of individual variation was observed among the six calves. It can be seen from Table 60 that the results suggest that passage

TABLE 60

Differences between animals in passage rates k_1 and k_2 (hr^{-1}) measured using Cr-EDTA or Cr-treated fish meal

Animal No	Body weight (kg)	Dry matter intake ($kg\ d^{-1}$)	Passage rate (hr^{-1}) Cr-EDTA k_1	Cr-EDTA k_2	Cr-treated fish meal k_1	Cr-treated fish meal k_2
1	165	4.50	0.0831	0.21	0.0507	0.17
19	162	4.56	0.0738	0.30	0.0572	0.16
22	168	5.02	0.0842	0.21	0.0431	0.16
12	173	5.03	0.0678	0.30	0.0599	0.17
26	153	4.45	0.0796	0.30	0.0579	0.17
6	166	4.34	0.0790	0.21	0.0456	0.17
Mean*	165	4.63	0.0779	0.26	0.0524	0.17

*SED ± Means

k_1 = 0.0089 ($P < 0.01$)

k_2 = 0.0380 ($P < 0.01$)

The model of Grovum and Williams (1973b) gave a good fit to the experimental data. Correlation coefficients for the log plot of faecal Cr against time (k_1) averaged -0.985 for Cr-EDTA and -0.957 for Cr-treated fish-meal. Full details are given in Appendix 28.

rate from the rumen was unaffected by body weight or dry matter intake over the range found in this trial.

DISCUSSION

The passage rate of protein supplements from the rumen may affect greatly the extent of degradation in the rumen (Ørskov and McDonald, 1979). Consequently, it is important to have information on the passage rate of fish silage supplement from the rumen to determine the effective degradability of fish silage protein.

A practical problem with the estimation of passage rate of protein supplement from the rumen has been the difficulty of obtaining representative samples of rumen fluid. Recently the problem has been made easier since it has been demonstrated that the passage rate of Cr-treated proteins from the rumen can be determined from concentration of Cr in the faeces (Elimam and Ørskov, 1981). They found that the mean passage rate of Cr-treated fish meal from the rumen of sheep, fed a pelleted diet, was 0.023 hr^{-1} when estimated from rumen samples and 0.024 hr^{-1} when estimated from faecal samples. The correlation coefficient between the rumen and faecal estimates was ($r = 0.99$). The work of Elimam and Ørskov (1984) and Bax and Offer (1983) with Cr-treated fish meal showed that 4% sodium-dichromate in the crude protein DM was adequate to render fish meal completely undegradable without affecting particle size distibution or density. This suggests that Cr-treated fish meal is likely to behave in a manner similar to the original protein supplement. For this reason this procedure was used in this experiment to calculate the particulate passage rate appropriate to fish meal. The complex Cr-EDTA was used in this experiment to estimate the rumen passage rate applicable to liquid fish silage. This complex has been used as a reference substance both in trace amounts labelled with ^{51}Cr (Downes and McDonald, 1964) and in larger amounts (non-radio-active) permitting chemical estimation (Binnerts et al., 1968). The only theoretical disadvantage of Cr-EDTA its slight absorption and subsequent excretion in the urine. In practice, this is not likely to be troublesome.

With both markers (Cr-EDTA and Cr-treated fish meal) the values for k_1 and k_2 were obtained from the slope of the regression of the natural logarithms of faecal Cr-concentration (Grovium and Williams, 1973b).

The faster passage rate for Cr-EDTA can be attributed to the fact that the liquid phase passes from the rumen faster than that of the particulate phase (Elimam and Ørskov, 1981 and Evans, 1981). Thus, soluble fish silage is likely to leave the rumen faster than fish meal because its passage is associated with the liquid phase. An increase in ruminal passage rate from 0.05 to 0.08 hr^{-1} will have little effect on effective degradability for proteins with rapid initial rates of degradation (such as grass silage, casein and untreated fish silage). Nevertheless, for proteins which are progressively degraded (such as linseed meal and formalin-treated fish silage) the effect will be greater. Examples are given in Table 10 (Ørskov, 1982).

There are very few estimates of passage rate of protein supplements from the rumen of the calf. Bax and Offer (1983) using similarly aged animals fed a grass silage diet supplemented with fish meal measured the passage rate of Cr-treated fish meal from the rumen. They reported a k_1 value of 0.0537 hr^{-1} which is similar to the mean value obtained in this experiment.

The results of this study indicate that for both solid and liquid phase markers, k_1 was not related to live weight (Cr-EDTA, $r = 0.33$ and Cr-treated FM, $r = 0.23$) or to dry matter intake (Cr-EDTA, $r = 0.28$ and Cr-treated FM, $r = 0.02$). This finding is in agreement with the result reported by Bax and Offer, (1983). However, a negative correlation ($r = -0.75$) was observed between k_1 for Cr-EDTA and k_1 for Cr-treated fish meal. Animals gave high liquid passage rates tended to give lower solid passage rates. This suggests that for a narrow range of live weights and intakes passage rate is independent of these factors, but is an innate characteristic of each calf.

The low variability between calves in passage rate for the Cr-treated fish meal marker is in good agreement with the results of the above workers who suggested that the variation between calves diminished with increasing calf age because of the full development of the reticulo-rumen. However, high variability in passage rate between cattle has been reported by Campling et al. (1961).

The quantitative significance of k_2 is somewhat uncertain. There is evidence that k_2 is a measure of passage through the caecum and colon as first kinetic apply to the elimination of marker from these organs (Grovum and Williams, 1973b).

CONCLUSIONS

Significant differences in passage rate from the rumen between liquid and solid phase markers were measured. This is of practical interest because a larger proportion of liquid fish silage protein (especially of formalin-treated fish silage) would be predicted to escape ruminal degradation due to the higher liquid passage rate. The results emphasise the importance of correcting for an appropriate passage rate when predicting the effective degradability of fish silage protein.

GENERAL DISCUSSION

The areas where fish waste is likely to be available in the U.K. are easy to identify. In the U.K. surplus fish and processing waste is mainly converted into fish meal at plants situated at the major fishing ports. Whilst there is little difficulty in utilising the quantities of suitable raw material in these areas, there are many smaller ports and scattered fishing communities far away from fish meal factories. It is not economical to transport small amounts of relatively low value fish and offal over considerable distances to the nearest factory for processing. It is also equally difficult to justify the large capital outlay necessary to build a local fish meal plant.

Dumping of fish waste can create problems both for the industry and for the local authority, and furthermore, involves the loss of material, which could be used to improve the profitability and viability of the local fishing and farming enterprises.

At present, large resources of fish protein are being discarded in the U.K. and other countries and there is no immediate method for utilising this waste as human food. These resources are by-catch fish, waste from processing, and accidental catches in excess of local processing capacity. The conversion into fish silage of such waste may be a feasible way of utilising the resources.

The present study may be considered as a continuation of the work with fish silage which has been continuing in the U.K. over recent years. In the present work acid fish silage products were prepared mainly from whiting waste which is locally available. The methodology used was simple and the product was cheap, odourless and could be applied in many parts of the U.K. where waste fish is available. Fish silage production is a flexible process since it can be carried out on a scale varying from a tonne or less, up to many tonnes. The fish silages were prepared with 2.5% formic acid and resulted in acceptable and stable products. The technical problems of production were few but it was felt that it would be necessary to research carefully the problems of converting laboratory scale production to the field scale. The low capital investment required for fish silage production and the flexibility of operation

makes the process attractive to many fisheries where fish meal is not commercially viable. Since the product is bulky, it is best used locally within a short distance of the point of manufacture. Also, there should be a potential market large enough to absorb the product. However, economically this will depend on the selling price.

Strøm *et al*. (1980) has reported that acid preserved fish silage, if stored for more than 3 months without frequent stirring, may develop surface mould. This must be avoided because mycotoxins (e.g. aflatoxin) could be produced. Provided the silage is stirred regularly, mould growth need not be a problem for at least 5-6 months (Gildberg and Raa, 1977). A mixture of propionic and formic acids (1.5% propionic + 1.5% formic) may provide effective control of mould growth (Gildberg and Raa, 1977). However, if the material is to be consumed quickly, it is not necessary to include expensive propionic acid. The factors influencing the amount of preservative acid are well known but for commercial production a safety allowance should be used.

From the results of the chemical analysis in this work, it is evident that the fish silage has potentially a high nutritive value. The product can be variable in composition (water, protein, oil and ash) depending on the raw material used and the season. *In vitro* incubations with SRF indicated that untreated fish silage appeared to be a highly available nitrogen source for rumen micro-organisms. This is due to the fact that fish silage protein contains a high proportion of amino acids and small peptides due to autolysis during the ensiling process. Treatment of fish silage with formalin (5 cm^3 kg^{-1} fish waste) reduced the *in vitro* rate of ammonia released to a level comparable to that of fish meal. Formalin treatment was also effective in reducing protein breakdown during autolysis. Thus, formaldehyde treatment would be expected to improve the nutritional value of fish silage as a ruminant feedstuff. Excessive treatment with formalin caused almost complete inhibition of autolysis and resulted in a product unsuitable for animal feeding.

The study demonstrated that fish silage can be used as a protein supplement in the diet of the ruminant calf (at an inclusion rate of 25% on a fresh weight basis) and will support acceptable growth rates. It was consumed by sheep at this inclusion level and

promoted digestibility values which were indistinguishable from fish meal or urea supplements. Fish silage depressed the feed intake of calves compared with supplements of urea or fish meal. This effect was greatest when the calves were less than 18 weeks old. After this stage the depression in intake and weight gain was less and the feed conversion efficiency and calculated kf values for calves fed fish silage were superior to urea but, less than fish meal.

It is known that fish silages often contain substances which retard intake and growth, particularly when fed at high levels. Possible intake inhibitors are oxidised fish oil derivatives or toxic amines (Hillyer et al., 1976 and Disney et al., 1978). The high content of highly unsaturated oil may reduce diet palatability and inhibit the activity of rumen microbes (Johnsen and Ekern, 1982) although the digestibility measurements suggest that this latter effect was not the case in the present study. There is evidence that the removal of the oil from fish silages improves animal growth and silage keeping quality (Johnsen and Ekern, 1981).

Thus, fish meal or urea supplements gave greater live weight gains and intakes than fish silage. However, comparison of efficiency terms (feed conversion efficiency or kf) showed that fish silage promotes growth of calves at efficiencies comparable to conventional supplements. The economic viability of the use of fish silage depends on the balance between the saving of feed costs and the reduced value of the product (due to decreased live weight gain).

Calculations using current prices showed that if the fish waste is cheap (less than £22 t^{-1}), then silage production is financially superior to the purchase of conventional supplements. In countries (or localities) where imported protein supplements are more expensive compared to the value of the end product then fish silage production for ruminant animal feeding appears to be economically viable.

FUTURE RESEARCH

Recommendations for future work are listed below:

a. There is need for more detailed information on the quantities of fish waste not used for fish meal in the U.K. Also more information is required on the composition of by-catch both on a monthly and annual basis. Species composition

of the by-catch is important, but also seasonal chemical composition should be studied, particularly as the level of oil influences the value of the final product.

b. There is urgent work needed to investigate the economics of production of fish silage at sea. It is possible that work on this subject could be very profitable due to the considerable fish waste usually thrown overboard from factory processing ships.

c. Production of fish silage on a commercial scale for ruminant feeding should be investigated. A pilot scale system sufficient to provide fish silage for a number of farms should be set up and the production problems, animal performance and economic viability should be carefully monitored. This study would allow investigation of the use of fish silage for a range of classes of ruminant stock fed a variety of basal diets. Problems associated with the transport and marketing of fish silage would be considered. Practical feeding systems to allow convenient farm use of fish silage needs to be developed.

d. The factors in fish silage which limit intake and performance needs to be fully investigated. Methods to control their formation and/or to remove them from fish silage needs to be developed.

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APPENDIX 1

Analysis of variance of the effect of formalin level on the true protein proportion in fish silage

Source of variation	D.F.	S.S.	M.S.	V.R(F)	Significance
Formalin level	5	165438.9	33087.8	185.713	**
Residual	12	2138.0	178.2		
Total	17	167576.9	9857.5		

APPENDIX 2

Analysis of variance of the effect of formalin level on fish silage dry matter

Source of variation	D.F.	S.S.	M.S.	V.R(F)	Significance
Formalin level	5	250.667	50.133	20.509	**
Residual	12	29.333	2.444		
Total	17	280.000	16.471		

APPENDIX 3

Analysis of variance of % nitrogen disappearance from nylon bags incubated in the rumen

a. Low levels of HCHO-treatment

Source of variation	D.F	S.S.	M.S.	V.R(F)	Significance
Sheep	2	2.355	1.178	0.546	NS
Formalin level	5	577.720	115.544	53.605	**
Time	5	16.051	3.210	1.489	NS
Sheep & Formalin level	9	17.229	1.914	0.888	NS
Sheep & Time	10	15.673	1.567	0.727	NS
Formalin level & Time	25	140.205	5.608	2.602	**
Residual	45	96.997	2.155		
Total	101	866.230	8.577		

b. High levels of HCHO-treatment

Source of variation	D.F.	S.S.	M.S.	V.R(F)	Significance
Sheep	2	62.43	31.21	0.942	NS
Formalin level	4	49903.77	12475.94	376.681	**
Time	5	868.90	173.78	5.247	*
Sheep & Formalin level	8	143.11	17.89	0.540	NS
Sheep & Time	10	290.01	29.00	0.876	NS
Formalin level & Time	20	675.03	33.75	1.019	NS
Residual	40	1324.83	33.12		
Total	89	53268.05			

APPENDIX 4

Analysis of variance of % dry matter disappearance from nylon bags incubated in the rumen

a. Low levels of HCHO-treatment

Source of variation	D.F.	S.S.	M.S.	V.R(F)	Significance
Sheep	2	11.063	5.531	0.736	NS
Formalin level	5	1202.795	240.559	32.023	**
Time	5	49.336	9.867	1.314	NS
Sheep & Formalin level	9	212.794	23.644	3.147	**
Sheep & Time	10	71.388	7.139	0.950	NS
Formalin level & Time	25	313.802	12.552	1.671	NS
Residual	45	338.042	7.512		
Total	101	2199.219	21.774		

B. High levels of HCOHO-treatment

Source of variation	D.F.	S.S.	M.S.	V.R(F)	Significance
Sheep	2	5.47	2.74	0.233	NS
Formalin level	4	27694.07	6923.52	588.629	**
Time	5	27.87	5.57	0.474	NS
Sheep & Formalin level	8	44.29	5.54	0.471	NS
Sheep & Time	10	86.34	8.63	0.734	NS
Formalin level & Time	20	233.25	11.66	0.992	NS
Residual	40	470.48	11.76		
Total	89	28561.76	320.92		

APPENDIX 5

Analysis of variance of effective degradability of fish silage incubated *in sacco* at 0.05 hr^{-1} passage rate

a. Low levels of HCHO-treatment

Source of variation	D.F	S.S	M.S	V.R(F)	Significance
Sheep	2	0.3333	0.1667	0.556	NS
Formalin level	5	78.6666	15.7333	52.444	**
Residual	10	3.0000	0.3000		
Total	17	82.0000	4.8235		

B. High levels of HCHO-treatment

Source of variation	D.F.	S.S.	M.S.	V.R(F)	Significance
Sheep	2	13.33	6.67	0.362	NS
Formalin level	4	7639.06	1909.77	103.698	**
Residual	8	147.33	18.42		
Total	14	7799.73	557.12		

APPENDIX 6

Analysis of variance of the effect of formalin treatment on kNH_3 (between treatment 1 and 6)

Source of variation	D.F.	S.S.	M.S.	V.R(F)	Significance
Formalin level	1	9.7092	9.7092	12.196	**
Residual	9	7.1647	0.7961		
Total	10	16.8738	1.6874		

APPENDIX 7

Analysis of variance of the effect of formalin treatment and time on *in vitro* ammonia production (as % of total-N) during 5 hr incubation

Source of variation	D.F.	S.S.	M.S.	V.R(F)	Significance
Formalin level	1	676.047	676.047	113.047	**
Time	5	5757.352	1151.670	192.626	**
Formalin level & Time	5	134.380	26.876	4.495	*
Residual	57	340.791	5.979		
Total	68	6909.566	101.611		

APPENDIX 8

Effective degradability of the complete diets used in the trial measured using the Dacron bag technique

Diet	Sheep No			Mean effective degradability % (dg)
	1	2	3	
CS and FS+	80	75	80	78
U	89	88	88	88
CSFM+	74	73	74	74
FM	74	69	65	69

+Degradability value of the basal diet without fish silage supplements.

APPENDIX 9

Health record and treatment

Date	Calf No.	Dose	Treatment
November	2	5 g/50 kg LW	Fulcin
"	5	"	"
"	10	"	"
"	13	"	"
"	17	"	"
"	19	"	"
"	20	"	"
December	1	"	"
"	2	"	"
"	3	"	"
"	4	"	"
"	5	"	"
"	6	"	"
"	8	"	"
"	16	"	"
"	18	"	"
"	21	"	"
"	27	"	"
"	28	"	"
January	11	"	"
"	22	"	"
"	25	"	"
"	30	"	"

APPENDIX 10

Mean live weight (kg) and live weight gain (kg) of the animals on each diet over the trial

Week	Diet									
	CS		FS		CSFM		U		FM	
	LW	LWG	LW	LWG	LW	LWG	LW	LWG	LW	LWG
6.11.1984	88.92	0.0	93.33	0.0	88.00	0.0	92.75	0.0	91.42	0.0
13	91.33	2.41	96.25	2.92	92.67	4.67	94.92	2.17	95.08	3.66
19	95.92	7.00	100.67	7.34	98.00	10.00	102.50	9.75	102.67	11.25
27	101.00	12.08	106.08	12.75	104.08	16.08	108.92	16.17	111.00	19.58
4.12.1984	104.50	15.58	111.67	18.34	110.75	22.75	113.50	20.75	117.25	25.83
11	109.00	20.08	115.33	22.00	115.83	27.83	120.50	27.75	124.33	32.91
18	115.33	26.41	123.17	29.84	123.25	35.25	127.50	34.75	129.58	38.16
24	119.25	30.33	125.92	32.59	128.42	40.42	132.50	39.75	135.17	43.75
31	124.75	35.83	132.42	39.09	136.75	48.75	139.83	47.08	142.92	51.50
8.1.1985	131.17	42.25	139.50	46.17	142.17	54.17	147.50	54.75	153.33	61.91
15	138.75	49.83	147.67	54.34	153.17	65.17	158.00	65.25	163.83	72.41
22	146.08	57.16	154.08	60.75	160.67	72.67	163.67	70.92	170.92	79.50
29	153.58	64.66	162.92	69.59	171.07	83.08	173.75	81.00	181.92	90.50
5.2.1985	159.92	71.00	170.83	77.50	179.17	91.17	180.58	87.83	188.33	96.91
12	168.58	79.66	178.75	85.42	187.75	99.75	187.33	94.58	196.58	105.16
19	178.83	89.91	189.08	95.75	197.83	109.83	198.00	105.25	208.75	117.33

APPENDIX 11

Total dry matter intake and performance of each animal over 0-8 weeks of trial

Diet	Calf No	Total dry matter intake (kg)	Live-weight gain (kg d^{-1})	Feed conversion ratios (kg DMI kg^{-1} LWG)
CS	1	157.26	0.70	4.68
	6	154.57	0.74	4.44
	12	169.461	0.73	4.86
	19	155.79	0.65	4.99
	22	160.13	0.64	5.21
	26	154.65	0.65	4.96
	Mean	157.70	0.69	4.82
FS	4	171.48	0.80	4.47
	9	181.83	0.77	4.93
	15	171.71	0.77	4.65
	17	160.82	0.72	4.68
	24	172.30	0.69	5.23
	30	157.29	0.75	4.40
	Mean	169.20	0.75	4.73
CSFM	2	181.82	0.83	4.59
	10	193.76	1.04	3.90
	13	160.86	0.87	3.88
	18	167.22	0.89	3.93
	25	144.53	0.91	3.32
	29	175.58	0.87	4.03
	Mean	170.60	0.90	3.94
U	5	198.51	0.93	4.46
	7	203.76	0.79	5.37
	11	183.72	0.88	4.37
	20	172.12	0.86	4.19
	21	195.39	1.03	3.94
	27	188.59	0.96	4.11
	Mean	190.30	0.91	4.41
FM	3	199.37	1.11	3.74
	8	180.63	1.08	3.50
	14	190.75	1.10	3.60
	16	160.76	0.80	4.18
	23	199.71	1.08	3.84
	28	173.18	0.82	4.42
	Mean	184.10	0.99	3.88

APPENDIX 12

Total dry matter intake and performance of each animal over 9-15 weeks of trial

Diet	Calf No	Total dry matter intake (kg)	Live-weight gain ($kg\ d^{-1}$)	Feed conversion ratios ($kg\ DMI\ kg^{-1}LWG$)
CS	1	193.68	1.12	4.13
	6	184.60	1.03	4.26
	12	205.14	1.12	4.35
	19	209.62	1.22	4.08
	22	216.27	1.22	4.21
	26	186.34	0.91	4.89
	Mean	199.30	1.10	4.32
FS	4	207.58	1.08	4.57
	9	235.28	1.32	4.26
	15	234.57	1.10	5.08
	17	216.31	1.22	4.23
	24	229.71	1.27	4.30
	30	193.75	0.98	4.70
	Mean	219.5	1.16	4.52
CSFM	2	260.47	1.30	4.77
	10	263.02	1.32	4.28
	13	236.87	1.42	3.97
	18	232.55	1.24	4.25
	25	213.27	1.16	4.39
	29	242.25	1.37	4.20
	Mean	241.40	1.30	4.354
U	5	261.81	1.10	5.67
	7	251.65	1.15	5.23
	11	243.45	1.02	5.68
	20	240.92	1.37	4.19
	21	254.92	1.06	5.27
	27	243.60	1.25	4.62
	Mean	249.40	1.16	5.19
FM	3	258.89	1.37	4.25
	8	249.95	1.40	4.24
	14	230.27	1.14	4.80
	16	231.92	1.15	4.80
	23	264.53	1.29	4.88
	28	246.22	1.28	4.48
	Mean	246.00	1.27	4.57

APPENDIX 13

Total dry matter intake and performance of each animal over 0-15 weeks of trial

Diet	Calf No	Total dry matter intake (kg)	Weight gain (kg d^{-1})	Feed conversion ratios (kg DMI kg^{-1} LWG)
CS	1	350.94	0.84	4.26
	6	339.16	0.84	4.13
	12	374.60	0.91	4.46
	19	365.40	0.86	4.32
	22	376.40	0.96	3.99
	26	340.99	0.80	4.36
	Mean	357.90	0.87	4.25
FS	4	379.07	0.86	4.48
	9	417.11	1.06	4.56
	15	406.28	0.92	4.49
	17	377.13	0.93	4.13
	24	402.01	0.96	4.27
	30	357.04	0.83	4.37
	Mean	389.80	0.93	4.38
CSFM	2	442.29	1.04	4.33
	10	456.78	1.17	4.00
	13	397.73	1.10	3.68
	18	399.77	1.08	4.05
	25	357.79	0.96	3.81
	29	417.83	1.06	4.03
	Mean	412.00	1.07	3.98
U	5	460.32	1.07	4.41
	7	455.41	1.02	4.57
	11	427.17	1.02	4.27
	20	413.04	1.05	4.02
	21	450.31	1.07	4.32
	27	432.19	1.05	4.20
	Mean	439.70	1.05	4.30
FM	3	458.25	1.22	3.83
	8	430.58	1.22	3.60
	14	421.02	1.09	3.94
	16	392.68	0.99	4.04
	23	464.25	1.18	4.03
	28	413.40	1.10	3.82
	Mean	430.00	1.13	3.88

APPENDIX 14

Analysis of variance of total dry matter intake calculated from regressions

Period 0-8 weeks*

Source	D.F.	S.S.	M.S	V.R	COV.E.F.	Significance
Feed	4	2275.4	843.8	6.570	0.973	**
Covariate	1	802.7	802.7	6.249	1.210	*
Residual	24	3082.7	128.4			
Total	29	7260.8	250.4			

Period 9-15 weeks

Source	D.F.	S.S.	M.S	V.R	COV.E.F.	Significance
Feed	4	10491.0	2622.7	13.434	0.973	**
Covariat	1	611.1	611.1	3.130	1.085	NS
Residual	24	4685.4	195.2			
Total	29	15787.5	544.4			

Period 0-15 weeks

Source	D.F.	S.S.	M.S	V.R	COV.E.F.	Significance
Feed	4	24082.2	6020.6	11.097	0.973	**
Covariate	1	2535.7	2535.7	4.674	1.147	*
Residual	24	13020.6	542.5			
Total	29	39638.5	1366.8			

*Intake, liveweight gain and feed conversion ratio data were subjected to covariance analysis using the pre-trial live weight as the covariate. This should eliminate any responses due to the slightly different initial liveweight and the effect was found to be significant in some cases. Therefore, it was decided to use the covariate corrected data throughout this analysis.

APPENDIX 15

Analysis of variance of daily live weight gain (kg) calculated from regressions

Period 0-8 weeks*

Source	D.F.	S.S.	V.S	V.R.	CON.E.F.	Significance
Feed	4	0.386805	0.096701	13.567	0.973	**
Covariate	1	0.016088	0.016088	2.257	1.050	NS
Residual	24	0.171062	0.007128			
Total	29	0.573954	0.019792			

Period 9-15 weeks

Source	D.F.	S.S.	V.S	V.R.	CON.E.F.	Significance
Feed	4	0.16587	0.04147	2.902	0.973	*
Covariate	1	0.00202	0.00202	0.141	0.966	NS
Residual	24	0.34289	0.01429			
Total	29	0.51078	0.01761			

Period 0-15 weeks

Source	D.F.	S.S.	V.S	V.R.	CON.E.F.	Significance
Feed	4	0.278534	0.069633	15.831	0.973	**
Covariate	1	0.008935	0.008935	2.031	1.041	NS
Residual	24	0.105566	0.004399			
Total	29	0.393035	0.013553			

*See Appendix 14

APPENDIX 16

Analysis of variance of feed conversion ratio calculated from regression

Period 0-8 weeks *

Source	D.F	S.S.	M.S.	V.R.	CONV.E.F.	Significance
Feed	3	4.4225	1.1056	7.802	0.973	**
Covariate	1	0.0259	0.0259	0.183	0.967	NS
Residual	24	3.4011	0.1417			
Total	29	7.8495	0.2707			

Period 9-15 weeks

Source	D.F	S.S.	M.S.	V.R.	CONV.E.F.	Significance
Feed	4	2.6673	0.6668	4.359	0.973	**
Covariate	1	0.0499	0.0499	0.326	0.973	NS
Residual	24	3.6716	0.1530			
Total	29	6.3887	0.2203			

Period 0-16 weeks

Source	D.F	S.S.	M.S.	V.R.	CONV.E.F.	Significance
Feed	4	1.02990	0.25747	8.074	0.973	**
Covariate	1	0.06570	0.06570	2.060	1.042	NS
Residual	24	0.76536	0.03189			
Total	29	1.86096	0.06417			

*See Appendix 14

APPENDIX 17

Analysis of variance of daily dry matter intakes

Source of variation	D.F.	S.S.	M.S.	V.R. (F)	Significance
Week	4	123.87515	30.96878	1025.552	**
Feed	4	11.47698	2.86924	95.017	**
Week & Feed	16	3.06351	0.19147	6.341	**
Feed & Calf	25	7.40125	0.29605	9.804	**
Residual	100	3.01972	0.03020		
Total	149	148.83659	0.99890		

APPENDIX 18

Analysis of variance of live weight gains

Source of variation	D.F.	S.S.	M.S.	V.R. (F)	Significance
Week	4	5.25207	1.31302	63.547	**
Feed	4	1.17750	0.29437	14.247	**
Week & Feed	16	0.86141	0.05384	2.606	**
Feed & Calf	25	0.82737	0.03309	1.602	N.S.
Residual	100	2.06623	0.02066		
Total	149	10.18457	0.06835		

APPENDIX 19

Part of a program to calculate energy and protein requirements
and efficienccy of utilisation of ME for growing calves
using ARC 1980

```
MEI=DMI*ME:REM ME INTAKE
MD=MEI/DMI:REM RATION M/D

UDPI=(1-DG)*CP*DMI:REM UDP INTAKE
RDPI=(DG)*CP*DMI:REM RDP INTAKE

REM TISSUE PROTEIN REQUIREMENT
P1=((5.9206*(LOG(WT)/2.302585))-6.76)*6.25:REM ENDOG URINARY PROT
P2=(.018*WT^.75)*6.25:REM PROT IN HAIR SCURF
P3=LWG*(168.07-(.16869*WT)+(.0001633*WT^2))*(1.12-(.1223*LWG)):REM PROT IN LWG
P4=P1+P2+P3:REM TISSUE PROT. REQ

REM UDP REQUIREMENT
UDPR=1.91*P4-6.25*MEI:REM UDP REQ
IF UDPR<0 THEN UDPR=0

REM RDP REQUIREMENT
RDPR=7.8*MEI:REM RDP REQ

REM ENERGY REQUIREMENTS

NEM=(.53*((WT/1.08)^.67))+.0043*WT:REM NE REQ FOR MAINT
EVG=(4.1+.0332*WT-.000009*WT*WT)/(1-.1475*LWG):REM NE OF 1KG LWG
NEAG=EVG*LWG:REM NE OF MEASURED LWG

KM=.019*MD+.503:REM EFFICIENCY OF UTILISATION OF  ME FOR MAINTENANCE.
KP=.0424*MD+.006:REM EFFICIENCY OF UTILILISATION OF ME FOR LWG AT L=2

REM CALCULATION OF PREDICTED LWG
P=KM*LOG(KM/KP):B=KM/(KM-KP)
R=B-1-(B/EXP((MEI*P)/NEM))
NEPG=R*NEM:REM NE PREDICTED FOR LWG
LWP=NEPG/(4.1+.0332*WT-.000009*WT*WT+.1475*NEPG):PREDICTED LWG

NER=NEAG/NEPG:REM NE ACTUALLY STORED / NE PREDICTED
MEM=NEM/KM:REM NE REQ FOR MAINT
MEP=MEI-MEM:REM ME AVAILABLE FOR LWG
PKF=NEAG/MEP:REM CALCULATED KF
```

APPENDIX 20

Analysis of variance of efficiency of utilisation ME for gain (kf)

Source of variation	D.F.	S.S.	M.S.	V.R.(F)	Significance
Week	4	0.217551	0.054388	8.281	**
Feed	4	0.40632	0.101579	15.467	**
Week & Feed	16	0.401742	0.025109	3.823	**
Feed & Calf	25	0.249320	0.09973	1.519	
Residual	100	0.656746	0.0065		
Total	149	1.931671	0.012964		

APPENDIX 21

Analysis of variance of NEAP values

Source of variation	D.F.	S.S.	M.S.	V.R. (F)	Significance
Week	4	1.13912	0.28478	10.013	**
Feed	4	2.28566	0.57141	20.092	**
Week & Feed	16	1.60945	0.10059	3.537	**
Feed & Calf	25	0.95560	0.03822	1.344	
Residual	100	2.84402	0.02844		
Total	149	8.83385	0.05929		

APPENDIX 22

Analysis of variance of dry matter digestibility

Source of variation	D.F.	S.S	MS.	V.R(F)	Significance
Period	4	6346.2	1586.6	2.723	NS
Sheep	4	1237.8	309.5	0.531	NS
Diet	4	308.2	77.1	0.132	NS
Residual	12	6991.5	582.6		
Total	24	14883.8	620.2		

APPENDIX 23

Analysis of variance of organic matter digestibility

Source of variation	D.F.	S.S	MS.	V.R(F)	Significance
Period	4	6496.6	1624.1	3.069	NS
Sheep	4	643.8	160.9	0.304	NS
Diet	4	271.0	67.7	0.128	NS
Residual	12	6349.7	529.1		
Total	24	13760.9	573.4		

APPENDIX 24

Analysis of variance of energy digestibility

Source of variation	D.F.	S.S.	M.S.	V.R(F)	Significance
Period	4	6451.4	1612.9	2.281	NS
Sheep	4	256.6	64.2	0.091	NS
Diet	4	1359.3	339.9	0.481	NS
Residual	12	8484.3	707.0		
Total	24	16551.8	689.7		

APPENDIX 25

Analysis of variance of *in vivo* digestible energy

Source of variation	D.F.	S.S	M.S.	V.R(F)	Significance
Period	4	4.9629	1.2407	4.000	*
Sheep	4	0.4280	0.1070	0.345	NS
Diet	4	1.8929	0.4732	1.526	NS
Residual	12	3.7224	0.3102		
Total	24	11.0062	0.4586		

APPENDIX 26

Apparent digestibility values of the experimental diets during the different periods of the trial

Sheep No.	Diet	Period	DM-digestibility (g kg^{-1})	OM-digestibility (g kg^{-1})	Energy-digestibility (KJ MJ^{-1})
89	CS	1	747	770	759
86	CS	2	755	794	775
83	CS	3	706	742	721
84	CS	4	776	806	794
90	CS	5	754	785	759
86	FS	1	716	744	742
89	FS	2	777	807	787
84	FS	3	750	787	742
90	FS	4	723	754	742
83	FS	5	781	814	787
83	CSFM	1	715	756	721
84	CSFM	2	770	805	774
90	CSFM	3	782	813	806
89	CSFM	4	771	804	780
86	CSFM	5	725	764	752
84	U	1	723	751	731
90	U	2	772	797	764
86	U	3	729	767	735
83	U	4	791	818	800
89	U	5	748	772	754
90	FM	1	719	758	723
83	FM	2	768	804	770
89	FM	3	733	762	711
86	FM	4	752	794	755
84	FM	5	744	781	764

APPENDIX 27

Analysis of variance of passage rates of Cr-EDTA Cr-treated fish meal through the digestive tract of calf

a. Ruminal passage rate (k_1 % hr^{-1})

Source of variation	D.F.	S.S.	M.S.	V.R(F)	Significance
Period	1	0.6120	0.6120	0.772	NS
Calf	5	0.5641	0.1128	0.142	NS
Marker	1	19.5330	19.5330	24.632	**
Residual	4	3.1719	0.7930		
Total	11	23.8811	2.1710		

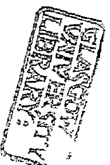
b. Post ruminal passage rate (k_2 hr^{-1})

Source of variation	D.F.	S.S	M.S.	V.R(F)	Significance
Period	1	4.08	4.08	0.285	NS
Calf	5	61.42	12.28	0.857	NS
Marker	1	234.08	234.08	16.331	**
Residual	4	57.33	14.33		
Total	11	356.92	32.45		

APPENDIX 28

Correlation Coefficient for the faecal excretion of Cr using model of Grovum and Williams (1973b)

Animal No.	Period	Marker Used	k_1	R_1	N_1	k_2	R_2	N_2
1	1	Cr-EDTA	0.0831	-0.986	9	0.2126	-0.980	4
19	1	"	0.0738	-0.987	13	0.2984	-0.939	3
22	1	"	0.0842	-0.976	11	0.2126	-0.980	4
12	2	"	0.0678	-0.976	9	0.2984	-0.939	3
26	2	"	0.0796	-0.997	13	0.2984	-0.939	3
6	2	"	0.079	-0.993	11	0.2126	-0.980	4
1	2	Cr-Fishmeal	0.0507	-0.975	8	0.1703	-0.966	3
19	2	"	0.0572	-0.950	13	0.1620	-0.979	4
22	2	"	0.0431	-0.958	9	0.1585	-0.995	5
12	1	"	0.0599	-0.943	9	0.1703	-0.966	3
26	1	"	0.0579	-0.977	11	0.1703	-0.966	3
6	1	"	0.0456	-0.936	10	0.1703	-0.966	3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