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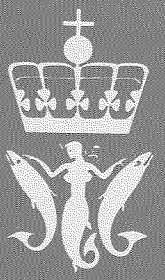

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EFFECTS OF PRE-DIGESTED PROTEIN INTAKE ON GROWTH AND MUSCLE METABOLIC PARAMETERS IN ATLANTIC SALMON *SALMO SALAR*

By

ALEXANDRA VON DER DECKEN[1]), MARIT ESPE[2]) and EINAR LIED[2])

[1]) The Wenner-Gren Institute for Experimental Biology, University of Stockholm, S-106 91 Stockholm, Sweden

[2]) Institute of Nutrition, Directorate of Fisheries, P.O. Box 1900, N-5024 Bergen, Norway

ABSTRACT

Atlantic salmon (*Salmo salar*) of 115-155 g body weight were fed for 49 days a diet containing high-quality fish meal protein of cod (*Gadus morhua*) muscle at a protein:energy level of 52% (diet A), or the same diet with the protein being pre-digested with pepsin for 6 h (diet B) or 48 h (diet C). Pre-digestion resulted in a pronounced decrease in proteins of mw > 60K, and a major increase in those of mw between 25K and 2K. Diet C caused a marginal non-significant decrease in specific growth rate and percentage weight gain. Metabolic parameters of the white trunk muscle were unchanged in RNA, DNA and glycogen content, showed a slight non-significant decrease of 3.5% in total protein and of 8.6% in proteins of sarcoplasmic origin. Myofibrillar proteins and myosin heavy chain as determined by the immunosorbent assay were unchanged. Acid proteinase activity/g wet weight increased significantly when feeding diet C, activity/mg DNA when feeding either of the pre-digested diets. The results show that extensive pre-digestion of feed proteins (diet C) marginally decreased growth and significantly affected protein accretion through an unchanged capacity for protein synthesis and an elevated level of protein degradation. From the results it is inferred that as a food component non-digested proteins are preferable over pre-digested ones.

INTRODUCTION

All fish species and especially carnivores have a high requirement for protein (Ketola, 1982). The amino acids of the intragastrically degraded proteins are precursor for different biological reactions, primarily the synthesis of new body proteins. They are also substrate in energy production (Cowey and Walton, 1989). The carbon skeleton of the deaminated amino acids are

oxidized in the citric acid cycle or converted to glucose and lipids. As dietary carbohydrates are relatively poorly utilized by fish the main nutritional energy sources are proteins and lipids (Cowey and Walton, 1989; Hemre *et al.*, 1989).

The aim of the fish farmer is to convert the feed into live fish at maximum intensity. Replacement in the feed of an optimal mixture of amino acids for the dietary proteins imposes less demands on the digestive system than would dietary proteins. However, in Atlantic cod (*Gadus morhua*) basal acid secretion is depressed by the administration of a mixture of L-amino acids (Holstein and Haux, 1982). The attained level of plasma amino acids seems related to the degree of acid secretion (Holstein and Haux, 1982). Gastric peptide hormones may be involved in the flow of digesta and stomach retention (see Ash, 1985). Their response to dietary proteins or amino acids may differ in this respect. When feeding an amino acid mixture a non-synchronous absorption of the free amino acids may be the reason for the diminished growth rate observed in Atlantic salmon (Espe and Njaa, 1991). In rats a dietary amino acid mixture is less efficiently utilized for muscle protein synthesis than are dietary proteins (Omstedt and von der Decken, 1974).

Pre-digested protein as a source of dietary nitrogen represents a situation between native protein and an amino acid mixture (Tacon and Jackson, 1985). Intragastric digestion may become more efficient by feeding the partially pre-digested proteins. In the present study high-quality fish meal protein was subjected to enzymic degradation prior to feeding Atlantic salmon (*Salmo salar*). Growth rate was marginally affected. Among the muscle metabolic parameters studied protein metabolism responded to the pre-digested food source.

MATERIALS AND METHODS

Materials

All chemcials were of the highest purity available, supplied by Sigma Chemical Co., St. Louis, MO, and Serva, Heidelberg, Germany. The standard fish food was obtained from Skretting A/S, Stavanger, Norway.

Fish and feeds

Atlantic salmon of between 80 g and 120 g body weight were used. Each tank of 1.3 m^3 water volume contained 40 fish. They were acclimatized to brackish water of a salinity of 16.9±1.9 g/L for 3 weeks and fed *ad libitum* a standard

diet (Skretting, Stavanger, Norway). The aquaria were supplied with running brackish water at 18-20 L kg min^{-1} and a temperature of 10.8±1.1°C. The fish were exposed to constant light. After the acclimatization period the fish were given the experimental diets, diet A, diet B or diet C (Table 1). In diet A the protein source was cod muscle meal; in diet B and C this protein source was digested with pepsin for 6 h and 48 h respectively. To digest the proteins pepsin was dissolved in distilled water and added to the ground cod muscle protein. The ratio was 200 g enzyme per 7 kg protein in a final volume of 40 L. The pH was adjusted to 4.2 with HCl. Incubation temperature was 27°C for 6 h and 48 h respectively. The reaction was terminated by rising the pH with NaOH. The suspension was concentrated by freeze-drying prior to preparing the diets (Table 1). After the acclimatization period the fish were

Table 1: Composition of the experimental diets[1]).

Ingredients (g x kg -1)	Diet A	Diet B	Diet C
Cod muscle [2,3])	528	0	0
Hydrolyzed cod muscle, 6 h	0	528	0
Hydrolyzed cod muscle, 48 h	0	0	528
Capelin oil (*Mallotus villosus*) [4])	185	185	185
Extruded wheat	205	205	205
Mineral mixture [5])	50	50	50
Vitamin mixture [6])	2	2	2
Gelatine	30	30	30

[1]) Energy value MJ x kg^{-1} diet: Protein and hydrolyzed proteins 9.5; fat 6.2; carbohydrates 2.6.
The values of 18.0, 33.5 and 12.5 KJ x g^{-1} were used to calculate the digestible energy of protein, fat and carbohydrates, respectively (Brett and Groves, 1979).

[2]) Molecular weight distribution of the feed protein (g x kg^{-1} total N):

Molecular weight/diet	Diet A	Diet B	Diet C
mw > 66 000	802	562	371
66 000 < mw > 25 000	11	3	1
25 000 < mw > 2 000	46	137	359
mw < 2 000	141	298	269

[3]) Toro, Bergen, Norway.

[4]) Norsildmel, Bergen, Norway.

[5]) Mineralmixture (g x kg^{-1} dry weight mineral mixture): $CaHPO_4$ x 7 H_2O 375, KH_2PO_4/300, NaCl 200, $MgSO_4$ 100, $FeSO_4$ x 7 H_2O 10, $ZnSO_4$ x 7 H_2O 10, $MnSO_4$ x 4 H_2O 2, $CuSO_4$ x 5 H_2O 0.4, KI 0.30. Dextrin was added 2to 1000 g.

[6]) Vitamin mixture (mg): Thiamine 143, riboflavin 143, pantothenic acid 143, Niacin 286, pyridoxin 571, biotin 357, folic acid 286, vitamin B_{12} 286, inositol 7 143, ascorbic acid 14 285, choline 78 571, α-tocopherol acetate 5 714, retinyl palmitate 24, cholecalciferol 4.

fed the individual diets. The feed was given by an automatic device at an amount of 0.5% of total biomass per day during 49 days. The fish were killed by a blow to the head and weighed. The white type of the epaxial muscle was dissected, sliced, wrapped into aluminium foil and frozen between 2 blocks of CO_2 and stored at -80°C (Lund and von der Decken, 1980).

Analytical methods

The food protein (N x 6.25) was determined by a modified Kjeldahl procedure (Crooke and Simpson, 1971); lipids by gravimetry of the ethylacetate extract of the food; ash by gravimetry after ashing for 24 h at 660°C and dry matter by gravimetry after drying for 24 h at 105°C. Carbohydrate content was the difference in weight between the sum of the above analytical results and the original weight of the food. The molecular weight distribution of the cod muscle proteins before and after enzymic digestion was determined by gel filtration (Table 1). The non-digested and pre-digested feed proteins were suspended in 0.1 M K-phosphate buffer, pH 6.8. The suspension was filtered and the filtrate passed through an UltraPac TSK G2 000 SW column with molecular weight separation between 500 and 60 000 (Pharmacia LKB Biotechnology, Stockholm, Sweden).

Preparation of muscle homogenate, separation into a sarcoplasmic and a myofibrillar fraction and the immunoassay have been described previously (von der Decken and Lied, 1992a,b; Nazar *et al.*, 1991). DNA was analyzed by a fluorescent method using salmon DNA as a standard (Setaro and Morley, 1976). Proteins were analyzed by the Commassie brillant blue method using bovine serum albumin as a standard (Bradford, 1976). RNA was determined after alkaline digestion of the perchloric acidprecipitate (Fleck and Munro, 1962). Glycogen was determined as described by Harris *et al.* (1974). The glycogen content was expressed as mg glucose g^{-1} wet weight of tissue. Acid proteinase activity was determined in the supernatant obtained after centrifugation of the muscle homogenate for 10 min at 1600 x g (Mommsen *et al.*, 1980). Haemoglobin was used as substrate and the tyrosine was analyzed by a fluorescent method (Ambrose, 1974). The results are expressed as mg tyrosine released from haemoglobin/h.

Statistical analysis

The results are expressed as means ±SEM. One-way analysis of variance and Newman Keuls test for multiple sample comparison were used to compare the 3 dietary groups with each other (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

After a feeding period of 49 days food intake per live weight gain was similar for the 3 groups (Table 2). Percentage average weight gain and the specific growth rate declined with the extent of pre-digestion of the feed protein without giving significant differences (Table 2). The protein content per g wet weight of muscle was similar for the 3 groups with a tendency of a decline after the extensive pre-digestion of diet C (Table 3). A separation of the muscle proteins into those of sarcoplasmic and myofibrillar origins emphasized a decline by 8.6% of sarcoplasmic proteins after feeding diet C (Table 3). It seems that the sarcoplasmic proteins rather than the contractile elements are subject to variation in protein levels during changes in environmental and physiological conditions. As compared with non-spawning fish adult Atlantic salmon after spawning show a decrease in the sarcoplasmic protein content

Table 2: Growth and food intake.

	Diet A	Diet B	Diet C
Initial body weight (g)[1])	156.6±1.2	118.3±5.1	116.0±3.8
Final body weight (g)[1])	209.3±1.4	156.4±5.6	150.1±3.9
Average weight gain (%)	33.6	32.2	29.4
Specific growth rate (% x day^{-1})[2])	0.59	0.57	0.53
Food intake (g)/live weight gain	0.85	0.84	0.85
Retained protein/eaten protein	0.42	0.42	0.44

[1]) Mean values ±SEM (n=40).

[2]) Specific growth rate, % x day^{-1} $= \frac{\text{ln final body weight - ln initial body weight}}{\text{Experimental days 49}} \times 100$

Table 3: Protein and myosin heavy chain content in white trunk muscle.

	Per g wet weight, mg protein			Myosin heavy chain (mg) per		
Diets	Total	Sarcoplasmic	Myofibrillar	mg protein	g wet weight	mg DNA
Diet A	133.38±1.31	65.80±1.58	67.45±2.07	0.240±0.023	32.04±3.08	39.05±3.83
Diet B	135.71±3.15	68.76±1.42	66.95±1.96	0.222±0.018	30.17±2.54	38.21±3.22
Diet C	128.79±3.38	60.14±1.68	69.08±2.29	0.225±0.015	28.94±2.08	36.08±2.60

The results are the mean values ±SEM (n = 8)

(von der Decken, 1992). Myosin heavy chain protein is representative of the myofibrillar proteins in muscle. Its content was between 22% and 24% of the total muscle proteins (Table 3). The level was that shown previously for muscle of Atlantic salmon (Nazar *et al.*, 1991; von der Decken, 1992; von der Decken *et al.*, 1992). Its content was unaffected by the dietary conditions.

RNA and DNA concentrations/g wet weight of muscle were similar for the 3 feeding groups (Table 4). The RNA content may be interpreted as an indirect measure of protein synthesis activities (Haines, 1973). The RNA/DNA ratio was the same for the 3 groups suggesting no dietary effect on the capacity for protein synthesis. In contrast to the protein distribution between the subcellular fractions the RNA level was higher in the sarcoplasmic than the myofibrillar part. A similar RNA distribution is seen in cod muscle (von der Decken and Lied, 1992a).

A pre-digestion of the dietary protein source would suggest a high efficiency of intragastric digestion of the remaining polypeptides and an increased amount of available amino acids for absorption. Excess would then be utilized for glycogen synthesis. In muscle the glycogen content was unaffected by the dietary conditions (Table 5), suggesting no additional amino acid load derived from the pre-digested proteins. An increase in glycogen content is noted when feeding excess of protein:energy levels making available amino acids for glucose and subsequent glycogen formation (von der Decken and Lied, 1992b). A fall in glycogen content is seen during extended time intervals of feeding frequency (von der Decken and Lied, 1992a).

A significant increase of 20% in acid proteinase activity g^{-1} wet weight of muscle was observed after feeding the extensively pre-digested diet C (Table 5). Per mg of DNA activity rose significantly for both diet B and C (Table 5). Based on the unchanged RNA content it was inferred that the capacity for protein synthesis was unaffected by the dietary conditions (Table 4). The total

Table 4: RNA and DNA content in white trunk muscle.

	Per g wet weight of muscle			
	RNA (mg)		DNA (mg)	Ratio
Diets	Sarcoplasmic	Myofibrillar		RNA/DNA
Diet A	0.791±0.048	0.640±0.029	0.820±0.016	1.774±0.060
Diet B	0.751±0.051	0.635±0.041	0.789±0.029	1.755±0.068
Diet C	0.786±0.051	0.620±0.049	0.802±0.041	1.753±0.024

The results are the mean values ±SEM (n = 8).

Table 5: Glycogen content and acid proteinase activity in white trunk muscle.

Diets	Glycogen[1]) per g wet weight	Acid proteinase activity[2]) per g wet weight	Acid proteinase activity[2]) per mg DNA
Diet A	9.00±0.18[a]	0.353±0.011[a]	0.430±0.012[a]
Diet B	9.07±0.25[a]	0.379±0.018[a,b]	0.480±0.020[b]
Diet C	8.69±0.38[a]	0.415±0.015[b]	0.517±0.025[b]

The results are the mean values ±SEM (n = 8). Columns with different superscript letters are significantly different, $P<0.05$.

[1]) Glycogen is expressed as mg glucose.

[2]) Acid proteinase activity is given as mg tyrosine released from the added substrate haemoglobin per hour.

protein content g^{-1} wet weight was decreased slightly and that of the sarcoplasmic fraction by 8.6%. The results in themselves gave no significant differences but were supported by the significant increase in acid proteinase activity. Thus, protein accretion was diminished through an elevated protein degradation activity when feeding the pre-digested diet C. The activity is inhibited by pepstatin (Woessner, 1972; von der Decken and Lied, 1992b), indicating the presence of cathepsin D (EC 3.4.23.5), an enzyme of lysosomal origin (Woessner, 1972).

When replacing dietary proteins by an amino acid mixture growth rate in Atlantic salmon is diminished (Espe and Njaa, 1991). The pre-digested proteins used here contained 27% to 30% of the nitrogen part as components with molecular weight below 2 000, while the non-digested proteins contained 14% (Table 1).

In conclusion, partially pre-digested feed proteins supported growth marginally less than the non-digested proteins when fish of 115 to 155 g body weight were used. Muscle performance was impaired by an increased protein degradation activity without an increase in protein synthesis as judged by the unchanged RNA content in muscle. Prolonged feeding periods might have emphasized the effects on growth and protein content in muscle. From an economical viewpoint non-digested proteins are preferred. As growing salmon will utilize both non-digested and pre-digested proteins, the choice should be the non-digested food source.

ACKNOWLEDGEMENT

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THE EFFECT OF STARVATION ON THE COMPOSITION OF ATLANTIC SALMON (*SALMO SALAR*)

By

ØYVIND LIE and INGVAR HUSE[1]

Institute of Nutrition, Directorate of Fisheries,
P.O. Box 1900, N-5024 Bergen.

[1]Institute of Marine Research, Austevoll Aquaculture Research Station, N-5392 Storebø.

ABSTRACT

Two groups of Atlantic salmon were starved for 78 days, the fish were weighed at start, after 35 days and 78 days of starvation and carcass, fillet and abdominal part of fillet were analysed for dry matter, protein and fat. The fish lost about 8% and 11% of the total weight during the starving period of 35 and 78 days, respectively. In the first period more than 50% of the weight loss was due to reduction in the weight of the viscera. The fat content in carcass, fillet and abdominal part of fillets declined in both groups during the periods of starvation, whereas minor changes were found in the protein contents.

INTRODUCTION

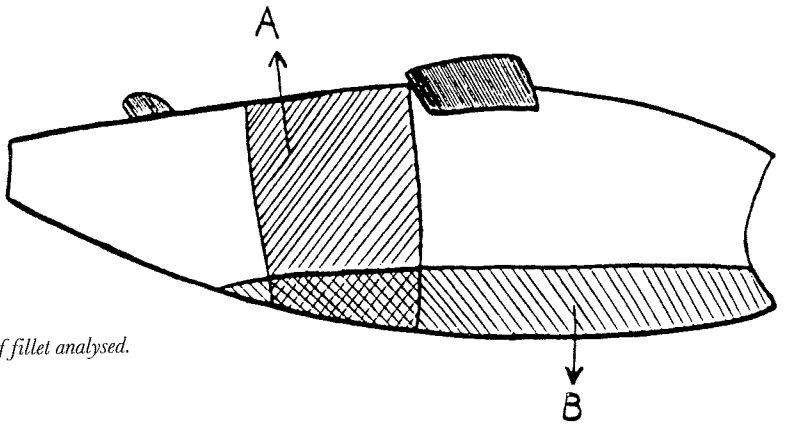
In the production of Atlantic salmon, high energy dry feeds are frequently used, resulting in large depots of visceral fat as well as high contents of fat in the fillet. This is undesirable from the consumers point of view and may be modified by the introduction of a fasting period, stimulating the mobilization and catabolism of lipids before slaughter, thereby giving a leaner fish.

Many fish species live through natural starvation periods and, in contrast to homoiothermous animals, can survive for months without food. Love (1980) has reviewed the utilization of protein, lipid, and carbohydrate reserves in fish during starvation. There is, however, a need for information on the utilization of the energy reserves in Atlantic salmon during periods of food deprivation. The aim of the present experiment was to study the effect of starvation on the proximate composition of carcass and fillet of Atlantic salmon.

MATERIALS AND METHODS

Two groups each of 330 Atlantic salmon, with initial weights of 2067g (±30) and 2642g (±37) respectively, were installed in net pens in the sea in November and left without feeding for 78 days. All fish were weighed at start, after 35 days and 78 days of starvation and a total of 20 fish from each group were collected randomly at each sampling date and stored at -20°C for further analysis.

Two pooled samples (n=10) from each group of carcass, fillet and the abdominal part of fillet (Fig.1) were ground and homogenized, and analysed for dry matter, protein and fat. Protein (N*6.25) was determined by the micro-Kjeldahl technique (Crooke and Simpson, 1971) and fat was extracted according to Lie *et al.* (1988a)



Parts of fillet analysed.

RESULTS AND DISCUSSION

The two groups of Atlantic salmon used in the experiment had been fed the same diet, but different feeding regimes gave differences in weight, group B being averagely 28% heavier than group A (Huse *et al.*, unpublished results). The weights of the fish at start and after the starving periods as well as the condition factors are given in Table 1. The weight loss in the two groups were 8.4 and 7.4%, respectively, of the total weight during the first period of 35 days, i.e. a mean weight loss of about 5 grams daily. More than 50% of the weight loss was due to a reduction in the weight of the viscera. The weight reductions of the gutted fish were less than 5% in group A and less than 4% in group B. The mean length of the fish increased with 0.6 cm in group A and 1.0 cm in group B during the first period of starvation (Table 1).

In group A there was a weight reduction of 11.6% after 78 days of starvation and in group B a reduction of 10.4%, whereas the mean length increased 0.6

Table 1. Weight, condition factor and weight of viscera of two groups of Atlantic salmon at start and after 35 and 78 days of starving.

	A			B		
	Initial	35 days	78 days	Initial	35 days	78 days
Weight, g	2067	1894	1827	2642	2449	2368
N	330	330	300	330	312	249
SEM	30	28	28	37	34	37
Length	547	553	559	577	587	589
SEM	2.3	2.3	2.5	2.3	2.4	2.7
C. fact.	1.23	1.10	1.02	1.35	1.19	1.14
SEM	0.01	0.01	0.01	0.01	0.01	0.01
Weight loss, g		173	240		193	274
Weight loss, %		8.4	11.6		7.3	10.4
Weight of viscera, g	245	158	133	296	189	184
Weight og viscera, % ...	11.9	8.3	7.3	11.2	7.7	7.8

cm and 0.2 cm, respectively (Table 1). However, the fish in group A had lost only 67g during the last 43 days compared to 173g the 35 first days, while the weight reduction in group B was 81g the last period compared to 193g in the first period. These results probably reflect that the metabolic rate was reduced as an adaptation to prolonged starvation. According to Love (1980) the reduction in the metabolic rate is mirrored in a steady decrease in oxygen consumption, while some of the reduction is due to reduced energy demand from lack of digestion and absorption of feed. Smith (1930) found that the oxygen consumption increased after 5 months of starvation, and he regarded this as evidence for an increase in protein catabolism. This condition has been regarded as a terminal feature of starvation in mammals, however, according to Love (1980) one should not expect this outcome in fish as the major energy source is amino acids. Also, older fish develop compensatory mechanisms and are thereby able to survive longer starving periods than younger fish (Love, 1980).

The weight loss of the viscera was markedly reduced during the last period of starvation, and the body-weight reduction during this period was probably due to the utilization of energy reserves in fillet. The main reduction in weight was therefore seen in gutted fish, which is unfavourable from commercial point of view.

Table 2. The proximate composition (%) of whole fish, fillet and abdomial part of filet of two groups of Atlantic salmon (*Salmo salar*) at start and after 35 and 78 days of starving.

	A			B		
	Initial	35 days	78 days	Initial	35 days	78 days
Whole fish						
Dry matter	34.8	34.1	33.2	37.3	36.8	35.8
Protein	16.6	16.0	16.2	15.2	16.6	16.4
Fat	15.1	14.8	14.2	19.0	17.1	16.6
Fillet						
Dry matter	32.5	32.1	29.7	34.4	33.6	32.3
Protein	19.1	18.8	18.7	17.8	18.7	18.4
Fat	11.3	11.4	9.3	14.0	13.0	11.9
Abdomial part of fillet						
Dry matter	41.0	38.8	36.7	43.4	42.2	40.6
Protein	16.1	16.6	16.2	14.8	14.9	15.7
Fat	23.2	20.5	19.2	26.3	25.2	23.3

The proximate composition of whole fish is given in Table 2. The protein levels seemed to be the least influenced by starvation. Some reduction was found in group A whereas an increase was found in group B during the fasting period. The fat level declined in both groups during the periods of starvation, the largest reduction was seen in group B during the first 35 days of starvation. In general fat seems to be the major energy source utilized during starvation of Atlantic salmon.

The proximate compositions of the fillet in group A were similar at start and after 35 days of starvation, whereas a drop in the fat content was found after 78 days of starvation (Table 2). There were some variations in the protein levels of fillets in group 2, while a linear decrease in the fat content was found during the starvation period. The reduction in the fat content gave a concomitant reduction in the dry matter content. The proximate composition for both groups at start, of whole fish as well as of fillet, was within the ranges reported for Atlantic salmon by Lie *et al.* (1988b). The differences in proximate composition between the fish in groups A and B reflect the differences in weight. These results are in accordance with previous studies (Lie *et al.*, 1988b; Waagbø *et al.*, 1991).

Atlantic salmon deposit surplus fat as visceral fat as well as in the fillets, and particularly in the abdominal part of the fillet. In this experiment, the abdominal part of the fillet had about twice the fat content compared to the

fillet sample (Table 2). The lipid content in the abdominal part of the fillet was reduced during the starvation, in group A most of the reduction was in the first period, whereas the opposite was seen in group 2 (Table 2). A concomitant reduction of dry matter content was found in both groups, while only minor differences were seen in the protein content during the starvation.

Prolonged periods of starvation are normal for many fish species during their life cycle, as during the spawning migration of Atlantic salmon. The effects of starvation on energy utilized are dependent on the species involved and the length of starvation period. For Atlantic salmon the lipid reserves were utilized during the initial period of starvation, and this has been reported for several fish species (reviewed by Love, 1980). According to a review by Cowey and Walton (1989) the liver lipid reserves of eel, rainbow trout and plaice are utilized first, followed by the reserves in muscle tissue. The present results show a utilization of visceral fat depot as well as fat and protein in the muscle. Jezierska *et al.* (1982) reported from an experiment with rainbow trout, starved for 48 days, that more lipid was mobilized from the perivisceral depot than from either liver or muscle. In contrast to mammals, teleosts probably do not produce ketone bodies during periods of starvation, and hydroxybutyrate was not detected in the blood of starved fish (Zammit and Newsholme, 1979; Hemre *et al.*, 1991). Also, 3-hydroxybutyrate dehydrogenase activity was not detected (Zammit and Newsholme, 1979).

The utilization of carbohydrate reserves (glycogen) during starvation seems to vary between species, and glyconeogenesis from amino acids complicates the picture. However, the glycogen levels in muscle tissue seems to be stable for a considerable time of starvation (Love, 1980; Hemre *et al.*, 1990, 1992).

Prolonged starvation involves increased proteolytic activity in the muscle, mobilizing amino acids from the muscle to ensure the utilization in more vital tissues. The amino acids are at that time also the major energy source for the fish (Cowey and Walton, 1989). However, 78 days of starvation of Atlantic salmon probably was a too short a period to have induced an extensive proteolysis of muscle tissue.

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EFFECTS OF DIETARY FORMATE AND ACETATE ON GROWTH AND LIPID DIGESTIBILITY IN ARCTIC CHARR, *SALVELINUS ALPINUS* (L.).

By

EINAR RINGØ

Present adress: The Norwegian College of Fishery Science, University of Tromsø
University of Tromsø Institute of Biology and Geology,
Dramsveien 201, N-9037 Tromsø, Norway.

SUMMARY

Anadromous Arctic charr, *Salvelinus alpinus* (L.), in triplicate groups of 35 fish each were reared for 84 days on diets with or without additions of 1% Na-formate or 1% Na-acetate. Dietary acetate significantly stimulated growth compared to fish fed the unsupplemented diet or the diet supplemented with formate.

Addition of 1% acetate to the diet affected the digestibility coefficients for both protein and lipid, and for the dietary fatty acids 14:0, 16:0, 18:0, 18:1, 20:1, 22:1 and 18:2 (n-6). Digestion coefficients for 16:1, 18:3 (n-3), 18:4 (n-3), 20:5 (n-3) and 22:6 (n-3) were not affected. Dietary formate had no significant effect on digestion of protein, lipid and dietary fatty acids.

INTRODUCTION

Arctic charr, *Salvelinus alpinus* (L.), are widely distributed in the Arctic Basin (Johnson, 1980) and occur as freshwater resident (stationary) and anadromous forms (Johnson, 1980; Nordeng, 1983). The fish has several favourable features for aquaculture: it grows at low temperatures, can withstand stocking densities of 200 kg/m^3 water (Reinsnes and Wallace, 1985), and it has an attractive appearance and is appreciated by consumers used to salmonid fish.

Fish silage used for feed purposes is preserved by added acids. The purpose of adding acids is to lower the pH sufficently to prevent spoilage caused by microorganisms. If only strong inorganic acids such as hydrochloric or sulphuric acids are used, the pH must be very low to prevent microbial spoilage. When weak organic acids e.g. formic or acetic acids are used in combination with inorganic acids, the undissociated molecule of the organic acid can pass the plasma membranes of the bacteria and thereby act antimicrobially.

Fish and fish offal may also be preserved by lactic acid bacteria (fermented silage) to produce lactic acid and acetic acid which lower the pH and inhibit microbial deterioation. Growth of lactic acid bacteria in fermented silage not only assists in preservation, but also add flawor to the products. A recent investigation demonstrated a stimulatory effect of a diet supplemented with lactate on the growth of Arctic charr (Ringø 1991 a). However, no information excists about the effect of dietary formate or dietary acetate on growth or lipid digestibility of Arctic charr.

Lipid digestibility in Arctic charr was studied by Ringø (1989; 1991 b). It was shown that dietary linoleic acid (18:2 n-6) (Ringø 1989) and salinity (Ringø 1991 b) affected lipid digestibility in Arctic charr.

This paper presents data on the growth of anadromous Arctic charr fed diets supplemented with 1% Na-formate or 1% Na-acetate, and gives data on digestibility coefficients for lipid and for the dietary fatty acids in Arctic charr fed these diets, and a diet with no addition.

MATERIALS AND METHODS

Fish and experimental conditions

Arctic charr, *Salvelinus alpinus* (L.), originating from the anadromous population which migrates into Storvannet, Hammerfest, Northern Norway were used, and triplicate groups of 35 charr (mean weight about 350 g) were held in self cleaning PVC tanks (diam. = 74 cm, h = 54 cm). The experimental period was from May to October. Fish were fed 1.2% of their body weight per day calculated according to biomass, assessed by weighing each forthnight. Aerated brackish water (Salinity = 8-10 g/L) at 8°C was continuously supplied.

Diets

The diets, a commercial dry pellet feed (Tess Elite Pluss 5.0 P, Skretting LTD) with or without addition of 1% Na-formate or 1% Na-acetate (Aldrich) were used. The diets were thoroughly mixed in a commercial mixer (Siemens) after the addition of formate or acetate and further supplemented with 2% soya-lecithin to ensure homogenous mixtures. Soya-lecithin was also added to the unsupplemented diet. New diets were prepared every four weeks.

Digestibility

Digestibility measurements were carried out on all 3x35 fishes per feeding groups after 91 and 105 days of feeding by using a faecal trap method (Figure 1). After anesthetizing the fish in 0.3% benzocaine, the stomachs were pumped

as described elsewhere (Santos and Jobling, 1988). Thereafter, the fish were starved for two days to ensure that the gastro-intestinal system was empty, and then the fish were given the experimental diets, 1.2% of their body weight spread over a twentyfour-hour period. Excess feed (feed not eaten) was removed regulary during this period. By using the faecal trap, exact amounts of feed eaten (feed intake) by the fish in each tank could be calculated.

Faeces was collected from each tank at two hours intervals over a period of 5 days, and faeces from each tank was treated separately.

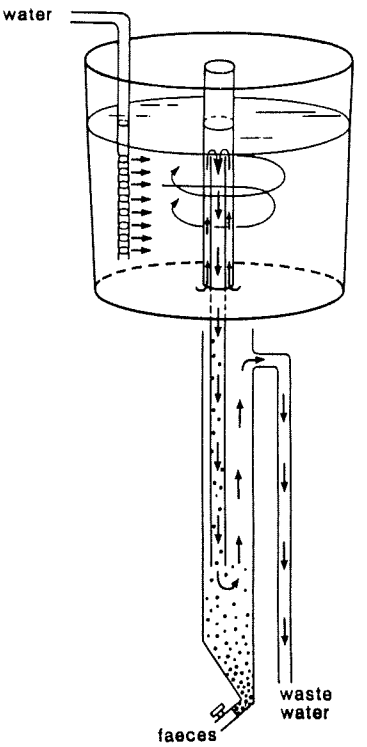
Digestibility (%) was determined according to the formula;

$$\text{Digestibility coefficient} = \frac{a - b}{a} \times 100$$

a = protein or lipid eaten; b = protein or lipid in faeces.

All data shown are grand means of 2 x 3 samples from each feeding group unless otherwise stated in text or tables.

Figure 1. Diagram illustrating the experimental design used for collecting faeces.



Chemical analyses

Dry matter was determined by drying samples for 48 hours at 110°C. Formic acid in the diets was determined enzymatically with kit no 979732 of Boehringer-Mannheim. Acetic acid in diets was determined as described by Ringø (1991 a).

Total lipid was extracted by the method of Folch *et al.* (1957) and stored at -80°C in hexane. Polar and neutral lipids were separated by thin-layer chromatography (TLC) on silica gel 60 plates as described by Tocher and Sargent (1984). The lipid extracts were saponified and esterified, and the fatty acids analysed by gas chromarography (GC) as described by Haug *et al.* (1988).

Protein contents in the diets were determined by a standard Kjeldahl procedure, multiplying the N content by 6.25. Carbohydrate was determined as described by Spiro (1966).

Specific Growth Rate (SGR) was calculated according to the formulae;

$$SGR = \frac{\ln Wt_1 - \ln Wt_0}{t_1 - t_0} \times 100$$

where Wt_0 = initial weight (g), Wt_1 = final weight (g), $t_1 - t_0$ = duration of experiment (in days).

Statistical test

To test possible differences in growth as a result of feeding the charr diet with or without addition of formate or acetate, a non parametric Mann-Whitney U test was used, while digestibility data were analysed by t-test. Significance was accepted at $P<0.05$ level.

RESULTS AND DISCUSSION

Triplicate analyses of formate concentration gave mean value of 11.0 mg/g dry weight for the diet supplemented with formate, and 0.1 mg/g dry weight for the acetate and unsupplemented diets. Concentration of acetate was 11.1 mg/g dry weight when acetate was supplemented to the diet and 1.8 mg in the other two diets.

Analytical data of the chemical composition (% of dry weight) and the fatty acid composition (%) of the dietary lipids in the diets are shown in Table 1. The high contents of 20:1 and 22:1 fatty acids in the diets are due to the use of marine fish oil in the diet.

Table 2 shows the effect of formate and acetate on the growth of Arctic charr. Fish were fed successfully on the acetate supplemented diet, and increased their weight from about 350 g to about 625 g, during the 84-days feeding

Table 1. Chemical composition (% of dry weight) of the diet and fatty acid composition (%) of dietary lipid. The values are mean of three diet samples.

Protein	49.1
Carbohydrate	24.5
Total lipid	16.9
Ash	7.5
Polar lipid	7.0
Neutral lipid	93.0
Fatty acids	
14:0	6.0
16:0	12.0
18:0	1.2
16:1a	6.7
18:1b	10.2
20:1	14.5
22:1	14.0
18:2 (n-6)	4.8
18:3 (n-3)	1.3
18:4 (n-3)	4.4
20:5 (n-3)	8.4
22:6	8.3

16:1a; 16:1 (n-7) + 16:1 (n-9)
18:1b; 18:1 (n-7) + 18:1 (n-9)

Table 2. Growth of Arctic charr, *Salvelinus alpinus* (L.), fed diets with or without supplement of 1% Na-formate or 1% Na-acetate over 84 days.

Dietary supplement	Initial wt.	Final wt.	Growth (%)	SGR
none	352.4	534.3	51.6	0.50
	348.4	548.6	57.5	0.54
	356.4	537.4	50.8	0.49
formate	344.5	568.2	64.9	0.60
	350.8	559.7	59.5	0.56
	342.7	551.2	60.8	0.57
acetate	350.0	631.0	80.3	0.70
	346.9	620.2	78.7	0.69
	347.4	623.2	79.4	0.70

Values are mean of 35 fish
SGR; Specific growth rate

experiment. This group had significantly ($P<0.05$) higher growth than the fish fed the unsupplemented diet (540 g). Fish fed the formate supplemented diet grew from about 350 g to about 560 g in the same period. However, there were no significant ($P<0.05$) differences in weight between fish fed formate supplemented diet and fish fed the unsupplemented diet at the end of the experiment.

The stimulated growth of fish fed the acetate supplemented diet may to some extent be explained by the higher feed intake (feed eaten) (Table 3), but enhanced digestibilities of dietary components might also contribute to the increased growth (Table 3 and 4).

Dietary formate had no significant ($P>0.05$) effect on the digestibilities of lipid and dietary fatty acids (Table 4). The digestibilities of the dietary fatty acids 16:1, 18:3 (n-3), 18:4 (n-3), 20:5 (n-3) and 22:6 (n-3) were not significantly ($P>0.05$) affected by the addition of acetate to the diet (Table 4). Significant ($P<0.05$) differences were, however, found in the digestibility coefficients of lipid, and of the fatty acids; 14:0, 16:0, 18:0, 18:1, 20:1, 22:1 and 18:2 (n-6) in fish fed the acetate enriched diet, relative to the two other treatment groups.

The increased lipid digestibility coefficients may be due to that acetate selectively affecting digestion or absorption of dietary fatty acids. Alternatively, a high level of acetate may affect the pH of the gastro-intestinal tract, and thereby increasing the efficiencis of the digestive lipases.

Austreng *et al.* (1979; 1980) observed effects of chain length and degree of unsaturation on the digestibility of fatty acids by rainbow trout *Oncorhynchus mykiss*. A similar pattern in the digestibility coefficients of individual fatty acids, was observed for Arctic charr (Ringø 1991b; 1989; the present study). In studies with rainbow trout, the digestibility of the n-3 fatty acids 20:5 and 22:6 was reported to be 100% (Austreng *et al.*, 1979). The digestibility of these two fatty acids in the unsupplemented diet was approximately 92%, but increased to approximately 97% by inclusion of acetate to the diet (Table 4). However, the enhanced digestibility of 20:5 (n-3) and 22:6 (n-3) was not significant.

The low digestibility of the long chain monoenes C_{20} and C_{22}, compared to the values for 20:5 (n-3) and 22:6 (n-3), may be due to lipase specificity in the hydrolysis of fatty acids from triacylglycerols (TAG) or to selective absorption of dietary fatty acids. Several studies have shown lipase fatty acid specificity in fish (for review see, Sargent *et al.*, 1989). On the other hand, Ringø and Olsen (1991) clearly demonstrated that, when methyl ester derivatives of dietary fatty acids were used to circumvent lipase specificities associated with TAG, 20:1 and 22:1, still accumulated in rectum contents compared to their counterparts in the (n-3) serie.

A noteworthy finding in this study is the relatively low digestibility coefficient (c. 86%) for 18:1 in the rearing groups fed the unsupplemented diet or the diet with added formate (Table 5). However, by feeding the fish a diet

supplemented with acetate the digestibility coefficient for 18:1 increased to c. 92%. The reason for this has not been elucidated and is a topic for further studies.

Table 3. Feed offer (1.2 % of body weight), feed eaten, and total faeces collected when Arctic charr were fed (A) unsupplemented diet, (B) diet supplemented with 1% Na-formate and (C) diet supplemented with 1% Na-acetate.

Treatment	Feed offer (g)	Feed eaten (g)	Total faeces (g)
A	226	150	35.0
B	231	155	36.5
C	265	188	30.0

Values are mean of 35 fish

Table 4. Apparent digestibility of (A) unsupplemented diet, (B) diet supplemented with 1% Na-formate and (C) diet supplemented with 1% Na-acetate fed to Arctic charr reared in brackish water.

Apparent digestibility	Experimental group			Statistical tests		
	A	B	C	A/B	A/C	B/C
Dry matter	76.7	76.5	84.0	n.s	*	*
Protein	90.9	90.8	95.7	n.s	*	*
Lipid	86.9	88.0	93.0	n.s	*	*
Dietary fatty acids						
14:0	88.6	89.7	95.4	n.s	*	*
16:0	79.0	80.1	89.4	n.s	*	*
18:0	70.7	71.6	81.3	n.s	*	*
16:1a	96.2	97.4	95.4	n.s	n.s	n.s
18:1b	85.7	86.6	92.2	n.s	*	*
20:1	83.8	84.9	90.5	n.s	*	*
22:1	80.3	80.9	88.5	n.s	*	*
18:2 (n-6)	81.0	82.0	87.0	n.s	*	*
18:3 (n-3)	94.6	95.3	96.0	n.s	n.s	n.s
18:4 (n-3)	95.5	96.7	97.3	n.s	n.s	n.s
20:5 (n-3)	92.2	93.0	96.3	n.s	n.s	n.s
22:6 (n-3)	91.7	92.6	97.2	n.s	n.s	n.s

16:1a; 16:1 (n-7) + 16:1 (n-9)
18:1b; 18:1 (n-7) + 18:1 (n-9)
*; $P<0.05$
n.s; not significant

The findings that dietary linoleic acid (18:2 n-6) (Ringø 1989), salinity, (Ringø 1991 b) as well as dietary acetate (the present study) affects digestibility of lipids in Arctic charr, indicate that digestion of dietary lipids in fish is complex.

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GROWTH RATE AND FEED CONVERSION FACTOR OF YOUNG HALIBUT (*HIPPOGLOSSUS HIPPOGLOSSUS* L.) FED SIX DIFFERENT DIETS.

By

BJØRN BJØRNSSON[1], GUNNAR SIGURTHORSSON[1], GRO-INGUNN HEMRE[2] and ØYVIND LIE[2]

[1]Marine Research Institute (MRI), P.O. Box 1390, 121 Reykjavk, Iceland
[2]Institute of Nutrition, Directorate of Fisheries,
P.O. Box 1900, N-5024 Nordnes, Bergen, Norway

ABSTRACT

Six groups of halibut, initial weight 2.5 kg, were fed the following diets: I. lean capelin (*Mallotus villosus* M.), II. fat capelin, III. lean and fat capelin, IV. moist feed from capelin silage, V. dry salmon feed and VI. moist feed from ground capelin, from 7.11.88 to 31.05.90. Most of the males became mature in the fall 1989, at a mean weight close to 4 kg. From 19.09.89 to 31.05.90 the mature males showed virtually no weight gain. For the first 3 months, the growth rates of the groups IV and V were substantially lower than for the other groups. These two groups showed a compensatory growth later on. Therefore, no significant differences in growth rate between the six groups were found for the whole experimental period. In the period prior to maturation of the males, the feed conversion factor was lowest, 1.0, for fish on diets I, II and III and highest, 3.0, for fish on diet IV. In the period subsequent to maturation the feed conversion factor increased substantially. A protein-sparing effect by fat was demonstrated by increasing the fat/protein ratio of a diet from 1.0 to 1.5.

INTRODUCTION

Atlantic halibut (*Hippoglossus hippoglossus* L.) is a new and promising candidate in fish farming. Its potential as an aquaculture species has been studied for less than 10 years. Much of this research has been carried out in Norway. The production of halibut fry has been the major obstacle to domestication. Thus the research effort has been concentrated on this problem. In the year 1985 the first two halibut fry survived in breeding trials in Norway. In the year 1991 approximately 150,000 halibut fry were produced in Norway and also a few thousands in Scotland and Iceland. Research into the propagation of halibut are also being done in Canada and the Faroe islands.

At the start of the present experiment (1988) a suitable diet had not been developed for the ongrowing of halibut. At the Marine Research Institute in Iceland (MRI) whole capelin coated with a special vitamin mixture had been used as feed with positive results. In Norway halibut fry were usually fed with a dry salmon feed, the immature halibut with a moist diet and the mature halibut with whole fish.

In Icelandic waters, capelin is harvested almost exclusively for the fish meal industry and is therefore a practical choice of raw material for halibut feed. For this reason all six diets used in the experiment were mostly or almost totally made from capelin or capelin products (meal, oil, silage). Three of the diets were whole capelin (vitaminized) varying in fat content from 6 to 15% and three of the diets were different types of processed diets (moist feed from silage, dry feed and moist feed from ground capelin).

This experiment was designed to elucidate a) whether formulated feed would be inferior to the more natural feed (whole capelin) with respect to growth rate and feed conversion in young halibut and b) to what extent fat exerted a protein-sparing effect.

MATERIALS AND METHODS

Fish

The MRI organized a collection program of immature halibut in Faxabay, SW Iceland, in the summer and fall of 1986 and 1987. Commercial fishermen fishing with Danish seine were paid a certain amount for each live halibut they brought to shore. Approximately 7000 halibut of 1-2 kg were collected. Roughly half of the fish survived and recovered fully. After a few months of adjustment the halibut were sorted by size. Most of them were used in a large scale experiment to study optimal stocking density, but a few hundred fish were reserved for other experiments, including the present feeding experiment. For several months prior to the experiment the fish were kept in a 6 m circular outdoor tank at a temperature near 7°C, and fed with whole capelin.

In 1988 MRI opened its own Aquaculture Research Station (ARS) 6 km west of Grindavik on the SW coast of Iceland. It is connected to the sea water system of a large commercial salmon farm (Iceland salmon Ltd.) which supplies ARS with sea water of constant temperature and salinity of respectively 7.1±0.2°C and 34-35g/L. The present experiment was carried out in six circular indoor tanks (2.9 m diameter) with water depth of 80 cm. The bottom of each tank was covered by 5 cm layer of fine dark sand.

The feeding experiment lasted for 570 days, from 7.11.88 to 31.5.90. At the start, 29 halibut weighing 2.4 kg on average were randomly selected, tagged

and put in each tank. Each fish was weighed and its length measured every 3-4 months. Prior to weighing the fish were starved for two days. No anaesthetics was required.

DIETS

The six groups of fish were fed to satiation six days a week the following diets: I. Lean capelin, II. Fat capelin, III. Lean capelin (Monday-Wednesday)/fat capelin (Thursday-Saturday), IV. Moist feed made from silage (14 mm salmon feed from Lysi hf.), V. Dry feed (12mm salmon feed from Ewos hf: Vextra EST93), VI. Moist feed made of ground lean capelin (60%), capelin oil (6%), capelin meal (12%), pellet mix (22%) from Ewos hf. (St58-35) and vitamin C (100mg/kg) were kneaded with a kitchen mixer; a fresh batch of feed was prepared each day. The fat/protein ratio was 0.5 for diets I, IV, V and VI, 1.0 for diet III, and 1.5 for diet II. The three formulated diets (IV, V & VI) contained about 10% carbohydrate in the matter and the capelin diets (I, II & III) less than 1%. The energy content of the diets varied from 5.0 MJ/kg (diet I) to 19.8 MJ/kg (diet V). For exact diet composition, see Table 1.

On Mondays and Thursdays a special mixture of vitamin mix (10%), choline chloride (5%), alginate (30%) and whole wheat (55%) was added to whole capelin (diets I-III), 25g per 1000g capelin. The vitamin mix (60g) contained the following vitamins: A: 262,500 IU, D_3: 52,500 IU, E: 5.25 g, K_3: 787.5 mg, Thiamine: 787.5 mg, Riboflavin: 1.31 g, Pyridoxin: 1.5 g, Folin: 262.5 mg, Niacin: 10.5 g, Pantothenic acid: 2.63 g, Biotin: 52.5 mg, Vitamin

Table 1 Proximate composition (g/100g of wet wt.), fat/protein ratio (g/g) and calculated energy content (MJ/kg) of the six experimental diets.

	I	II	III	IV	V	VI
Dry matter	22.4	0.5	26.9	65.9	95.6	51.7
Protein	11.8	10.1	10.8	28.7	41.5	24.0
Fat	6.1	15.3	11.2	13.4	22.4	11.6
Carbohydrate	0.1	0.1	0.1	7.3	10.4	4.9
Ash	1.8	1.5	1.6	5.8	7.4	4.2
Fat/protein ratio	0.5	1.5	1.0	0.5	0.5	0.5
Energy content[a]	5.0	8.3	6.8	12.9	19.8	10.8

[a] The conversion values used: 22.2 MJ/kg protein, 39.3 MJ/kg fat and 17.6 MJ/kg carbohydrate.

B_{12}: 1.05 mg, C: 15.75 g, Inositol: 21.24 g. The mixture was added to whole capelin just before feeding.

The fish were hand fed once a day except those receiving diets IV and V which were offered food twice a day because of less appetite on those diets. To ensure successful weaning to dry feed the pellets were frequently coated with moist feed (diet VI) for the first three weeks of the experiment. A special care was taken not to overfeed. At the end of each day any uneaten capelin was removed and its weight subtracted from the amount fed. The number of uneaten food particles for diets IV-VI were counted and from their average weight the uneaten portion was estimated and subtracted from the amount fed.

CHEMICAL ANALYSIS

At the start of the experiment a total of 10 fish were randomly selected for analysis of dry weight, protein, fat and ash. After 7 months 5 fish and at the end of the experiment 10 fish from each group were randomly selected for chemical analysis. The chemical analysis was performed by the Institute of Nutrition, Directorate of Fisheries, Bergen; those results will be presented more fully in a separate contribution.

Whole samples of fish and feed samples taken at the start and the end of the experiment were analysed for proximate composition. Protein (N x 6.25) was analysed according to Crooke and Simpson (1971) and fat was measured gravimetrically using ethyl acetate extraction. Ash was determined gravimetrically in dry matter samples ashed at 550°C for 20 hrs. Feed samples were also analysed for available carbohydrate (Hemre *et al.*, 1989).

CALCULATIONS

Specific growth rate (SGR) was calculated as % of body weight per day according to the formula:

$$SGR = 100(\ln W_2 - \ln W_1)/(t_2 - t_1)$$

where W_1 and W_2 is the weight (kg) of fish at weighing days t_1 and t_2. Food conversion (C_d) was calculated on a dry weight basis:

$$C_d = I_t D/(W_2 - W_1)$$

where I_t is the total food intake (kg) in the growth period and D is the fraction dry matter in the feed.

Protein Efficiency Ratio (PER) is defined as gain in weight(g)/g protein eaten.

STATISTICS

For compariceon of two tanks t-test was used, while analysis of variance (ANOVA) was used for comparison of all groups.

PALATABILITY EXPERIMENT

At the end of the experiment five halibut fed lean capelin (diet I) and five receiving fat capelin (diet II) were used in a palatability trial. Two steaks were cut from each fish and marked with a plastic tag. A total of ten Icelandic families were given two fresh steaks, one from each of the two experimental groups. The families were instructed to cook the halibut according to their choice provided that both steaks were cooked in the same way. Each family filled out a questionnaire about the preference of each family member, his/her age etc.

RESULTS

In March 1990 44% of the fish were mature males, most but not all of the immature fish were females. The percentage of mature males varied from 29% (diet II) to 58% (diet III) and their mean weight from 3.5 to 4.2 kg. The growth data were grouped as mature males and as immature fish (mostly females). For convenience these two groups are referred to as males and females.

For the females, no difference in growth was seen between groups I, II, III and VI (Fig.1). The growth of the fish fed silage (diet IV) and dry feed (diet

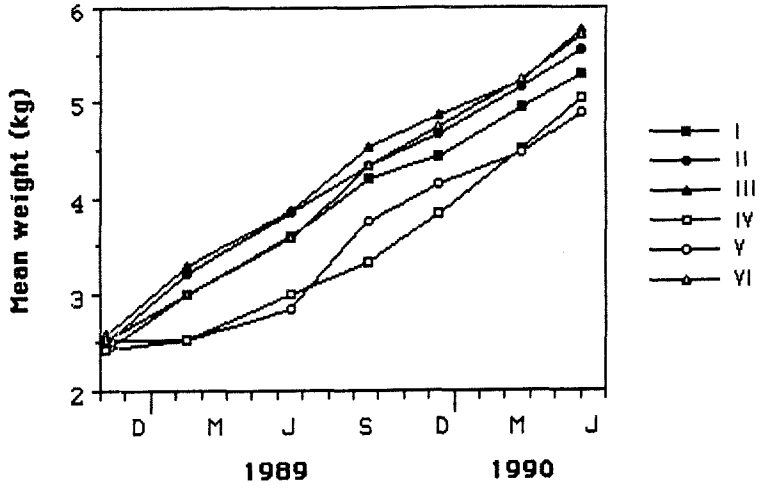

Figure 1.
Growth of immature fish (mostly females) fed six different diets.

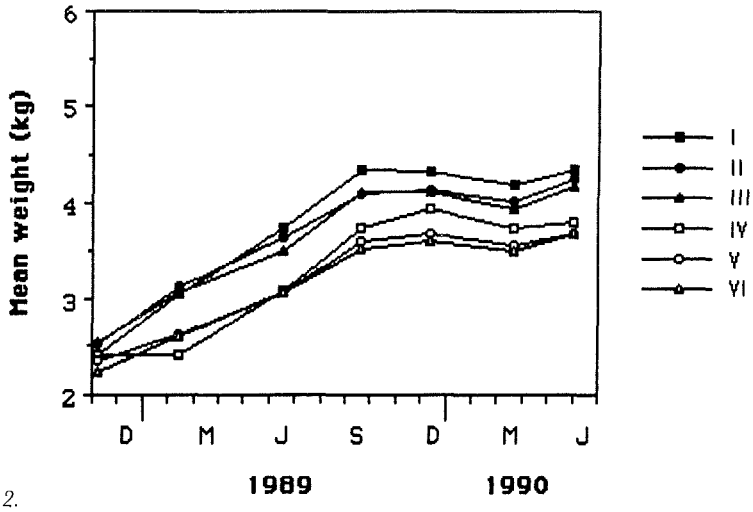

Figure 2.
Growth of males fed six different diets.

Table 2 Initial and final weight (kg), specific growth rate (SGR %/day),specific feed intake (SFI %/day), feed conversion factor (C_d = kg dry wt. of feed/kg gain in weight) and protein efficiency ratio (PER) for the six groups of fish as calculated for the whole experimental period.

		I	II	III	IV	V	VI
Initial weight:	Mean	2.42	2.51	2.57	2.49	2.40	2.40
(n=29)	SD	0.33	0.47	0.35	0.38	0.38	0.54
Final weight:	Mean	5.30	5.55	5.76	5.04	4.90	5.71
(immat. fish)	SD	1.46	1.56	1.11	1.06	1.25	1.47
	n	13	17	10	15	14	11
Final weight:	Mean	4.35	4.24	4.16	3.80	3.68	3.68
(mat. males)	SD	1.16	0.65	0.88	0.81	1.03	0.74
	n	11	7	14	9	10	13
SGR:	Mean	0.13	0.14	0.14	0.12	0.11	0.14
(immat. fish)	SD	0.03	0.03	0.02	0.03	0.05	0.04
SGR:	Mean	0.10	0.09	0.08	0.08	0.08	0.09
(mat. males)	SD	0.03	0.04	0.02	0.02	0.05	0.03
SFI		0.64	0.41	0.46	0.50	0.17	0.35
C_d		1.21	1.01	1.14	2.96	1.56	1.59
PER		1.57	3.00	2.19	0.78	1.48	1.35

V) was initially poor. A compensatory growth was, however, observed later. The males were initially of similar size as the females and grew similarly for the first three growth periods (10 months) (Fig. 2). However, from September 19, 1989 to May 31, 1990 there was virtually no growth of the mature males.

The mean specific growth rate for the whole experimental period was 11 and 16% lower for both males and females on diets IV and V relative to the other diets (Table 2). However, this difference was not statistically significant.

Comparison of the specific feed intake (SFI) (Table 2) and the energy content of the feed (Table 1) show that the intake was to a large extent regulated according to the energy content of the feed. The highest SFI (0.64) was found for the diet with the lowest energy content (diet I: 5.0 MJ/kg) and the lowest SFI (0.17) was found for the diet with the highest energy content (diet V: 19.8 MJ/kg).

The feed conversion factor (C_d) was much lower for whole capelin(1.0-1.2) than for the formulated feeds (1.6-3.0). Prior to maturation of the males C_d was 0.9-1.0 for diets II and III and 3.0 for diet IV. Except for diet IV, C_d was considerably higher in the last three growth periods than in the first three due to maturation of the males (Fig. 3).

The Protein Efficiency Ratio (PER) of the whole capelin diets (I, II & III) was substantially higher than for the formulated diets (IV, V & VI) (Table 7). PER increased with increasing fat content of the whole capelin.

The experimental setup made it possible to judge whether lean or fat capelin was preferred by the halibut (diet III). These fish were fed lean capelin on Mondays, Tuesdays and Wednesdays and fat capelin on Thursdays, Fridays and Saturdays. A higher consumption of fat than of lean capelin was observed in all growth periods. For the whole experimental period the intake

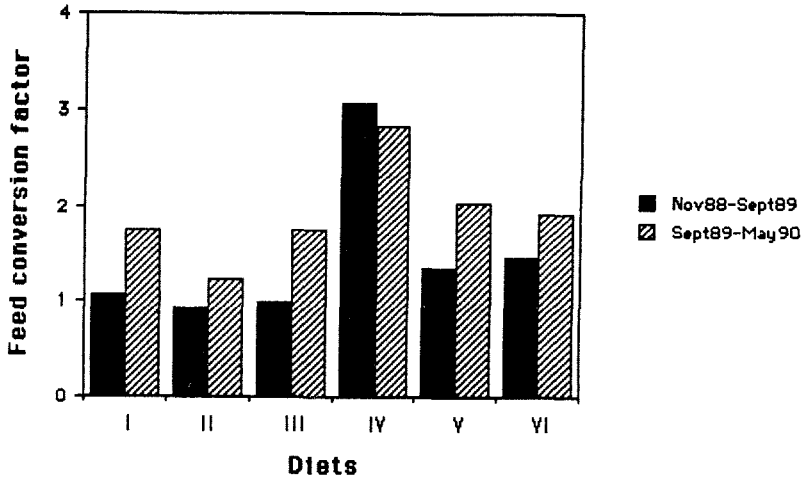

Figure 3.
Feed conversion factor for the six diets, before and after maturation of the males.

Table 3 Proximate composition (g/100g) of whole fish initially and at the end of the experiment for halibut fed six different diets.

	Initially	I	II	III	IV	V	VI
Dry matter	32.4	35.2	36.8	37.2	34.6	33.9	34.2
Protein	15.9	16.6	15.4	15.8	16.3	16.6	16.3
Fat	11.7	15.1	18.2	17.9	14.4	14.2	14.4
Ash	1.9	1.6	2.0	1.7	2.2	1.8	2.0

of fat capelin was 25% higher than that of lean capelin, corresponding to a 106% higher intake in terms of energy.

During the experimental period the fat content of the fish increased from 11.7% at the start to 14.2-18.2% at the end, with a positive correlation between the fat/protein ratio of the diet and fat content of the fish (Table 3). No marked difference in protein content was seen except for the somewhat low values in the fish receiving fat capelin (15.4%).

No consumer preference was found for halibut fed lean compared to fat capelin. For the 35 family members, 5 found no difference, 13 preferred halibut getting lean capelin and 17 preferred halibut getting fat capelin.

DISCUSSION

At the beginnig of the experiment it was observed that the halibut disliked two of the diets, the silage feed (diet IV) and the dry feed (diet V). This observation was further confirmed by the low food intake and poor growth rates of these groups during the first growth period (Figs. 1 & 2). At the beginning of the experiment the halibut took these food particles readily but spit them out once they were in the mouth, indicating a low palatability of these two diets.

During the first few weeks the halibut tried to chew the dry feed before they spit it out. Sometimes they would take the food pellet in the mouth and spit it out several times before swallowing or rejecting it. Perhaps the halibut found the dry feed too hard and perhaps too small, each pellet weighing only 1 g (0.03% of the fish weight). However, it cannot be ruled out that the halibut disliked the taste of the pellet. After several weeks the halibut stopped chewing and spitting out the dry feed and took it with ease both in the water and from the bottom. The whole capelin was accepted readily from the start.

The halibut pressed the silage feed (diet IV) in its mouth before swallowing it. This mode of feeding caused a large part of that diet to be lost between the gillrakers as small particles. The halibut maintained this mode of feeding until

the end of the experiment, accounting for the much higher food conversion of this diet compared to any of the other diets (Fig. 3). This resulted also in a high organic load and massive production of H_2S in the sand, which was cleaned at the time of each weighing. Apparently the dry feed caused much less pollution than the two other formulated diets. No signs of pollution were seen when using whole capelin. It is concluded that normal silage feed is not suitable as feed for the ongrowing of halibut due to poor pellet quality and great pollution.

The compensatory growth seen in groups IV and V is in accordance with the results with cod and many other animals (Pedersen and Jobling, 1989), in which previously fasted or poorly fed animals showed a marked growth spurt on return to good feeding conditions. In the present experiment compensatory growth did not fully compensate for the initially slower growth.

The observed differences in food intake between the six diets, varying greatly in terms of energy content, may indicate that halibut, like many other fish species, regulate their food intake according to their energy needs (Rozin and Mayer, 1961; Brett and Groves, 1979; Bromley, 1980b).

The feeding of fat versus lean capelin (fat/protein ratio 1.5 versus 0.5) did not result in any loss in appetite, decrease in growth, increase in feed conversion factor, or increase in mortality. These results are in accordance with results for juvenile and young halibut fed a diet with a fat/protein ratio varying from 0.2 to 1.0 (Berge and Storebakken, 1991). However, Hjertnes and Opstvedt (1989) found that growth rate of juvenile halibut (10-150 g) decreased with an increase in fat/protein ratio from 0.4 to 0.6. In their diets the fat and the metabolizable energy content was kept constant while protein was replaced by cabohydrate. Thus, their findings may as well be interpreted as poor utilization of carbohydrate by the juvenile halibut. For comparison, normal formulated feed for salmon has a fat/protein ratio of 0.5. For this species better growth and survival was found with an increase in fat/protein ratio from 0.4 to 0.6 (Austreng, 1986).

Although the fat/protein ratio of the diet had significant effects on the fat content of the halibut (Table 1 & 3), the fat content of the fish at the end of the experiment was high for all diets (14.2-18.2%) suggesting that the optimal diet of halibut may contain a high fat/protein ratio.

A protein-sparing effect by fat was clearly demonstrated in the present experiment, as in many other experiments with several fish species (Lee and Putnam, 1973; Adron *et al.*, 1976; Bromley, 1980a). The present experiment showed that halibut fed a diet with a fat/protein ratio of 1.5 utilized each g of protein better than if fed a diet with a fat/protein ratio of 1.0 (Table 2). The practical implication is that less protein relative to fat is required for the ongrowing of halibut than is considered optimal for many other species of fish

such as salmon (Austreng, 1986) and cod (Lie *et al.*, 1988). Since fat is a cheaper energy source than protein it is likely that relatively inexpensive feed can be developed for the ongrowing of halibut.

It must be pointed out that the mean growth rates observed in the present experiment, 0.13 %/day for the females (Table 2), were lower than that observed in a density experiment carried out in large tanks (8m diam.) by MRI in Iceland. For the females at the lowest densities (10-20 kg/m^2 and comparable mean weights the growth rates were close to 0.20 %/day calculated for a whole year. Although the two experiments differed in many ways it is possible that the full growth potential of the 2-6 kg halibut was not attained due to relatively small tank size (3m) used in the present experiment. A similar effect was found for 1-3 kg halibut by Berge and Linseth (1989) and Hamre (not published) who observed an increase in growth rate after transporting a few 7 kg halibut from 3m tanks to a 7m circular tank.

CONCLUDING REMARKS

The estimated feed cost to produce 1 kg of halibut in the present experiment was lowest for diet II ($1.80), second lowest for diet III ($2.16) and third lowest for diet V ($2.24). Since there was no indication of consumer dislike for the halibut fed fat capelin (diet II) it is concluded that the optimal diet for the ongrowing of halibut may have a relatively high ratio of fat to protein, at least in the range 1.0-1.5. Presently, it seems that whole fat capelin, supplemented with vitamins, has some advantages relative to the formulated feeds tested as a diet for young halibut: low cost, high palatability, good growth, low feed conversion factor and minor pollution.

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THE PROTEIN VALUE OF FISH SILAGE PREPARED FROM CAPELIN STORED UNDER DIFFERENT CONDITIONS BEFORE ENSILING. EFFECT OF STORING THE SILAGES FOR ONE YEAR.

By

MARIT ESPE and HERBORG HAALAND[1]
Institute of Nutrition, Directorate of Fisheries
P.O.Box 1900, Nordnes, N-5024 Bergen, Norway

[1]SINTEF Applied Chemistry, N-7034 Trondheim, Norway.

ABSTRACT

Eight silages were made from capelin (*Mallotus villosus*) which was stored under different time and temperature conditions prior to ensiling. PER values were obtained in experiments with rats when the silages had been stored for 2 and 6 weeks and for 3, 6 and 12 months. Only small differences were observed in the PER values until 6 months of storage, thereafter all PER values except that from cooked raw material declined. Some of the silages were run in nitrogen balance experiments with rats when freshly prepared. The silage made from newly thawed raw material showed somewhat higher utilization compared to the others tested. No differences in protein digestibilities with neither rats nor mink could be detected.

INTRODUCTION

In the search for quality criteria for fish silages 8 formic acid silages prepared from differently treated capelin (*Mallotus villosus*) raw material were analysed for chemical parameters during storage at 10-12°C for one year (Haaland *et al.*, 1990). It was concluded that of the parameters considered only total volatile nitrogen (TVN) and the amines cadaverine and tyramine seemed to be useful to characterize the quality. It was therefore of interest to study, with these silages of known history, whether differences in chemical parameters were reflected in quality differences as measured by protein utilization parameters. Two of the silages, one prepared from fresh capelin and one from cooked capelin have been reported on previously (Espe *et al.*, 1992a) in a study

on the effect of the degree of autolysis on the protein quality. Raw material which was not ensiled was included in that study.

In the present communication results from all the 8 silages are reported included the two reported on by Espe *et al.* (1992a). They served as controls.

MATERIALS AND METHODS

The silages were prepared as described by Haaland *et al.* (1990). The raw material was capelin (*Mallotus villosus*) frozen fresh from the same batch. Three silages were prepared from thawed capelin stored at 10-12°C for 3, 5 and 7 days (MT-3, MT-5 and MT-7), and three silages were prepared from thawed capelin stored at 2°C for 5, 10 and 14 days (KJ-5, KJ-10 and KJ-14) prior to ensiling. Two control silages were prepared from thawed capelin directly (FRS) and from cooked thawed capelin (VB). All silages were stored at 10-12°C for one year. PER determinations were done after storage for 1 and 6 weeks, and for 3, 6, and 12 months as described by Espe *et al.* (1992a). During the last five days of the PER-experiment faeces were collected for the determination of apparent digestibility (AD) of protein using Cr_2O_3 as the indicator substance (Rosenlund and Njaa, 1982).

New portions of the silages FRS, MT-5, KJ-5 and VB were prepared from the same batch of capelin, which were kept frozen as described above. With these newly produced silages a nitrogen balance experiment with rats was performed as described by Espe *et al.* (1989). These silages were not analysed, but it was assumed that they were similar to the others.

Chemical analyses were performed as described by Espe *et al.* (1989, 1992a) except for the amino acid analyses which were done on a Waters Amino acid Analyzer System (PicoTag) after prederivatisation with phenyl-iso-thiocyanate (PITCH).

The results were evaluated by two-way analyses of variance and by subdividing treatment sum of squares into single degrees of freedom (Snedecor, 1946).

RESULTS

Analytical data of the raw materials, of the freshly prepared silages and of silages stored for one year are shown in Table 1. Analyses of the freshly prepared silages may give a better picture of the raw materials than analyses of the raw materials. This is because during handling of the raw material for analysis, NH_3-N and amines may continue to be formed for some time, in the silages this will be inhibited by the low pH. In Table 2 are given amino acid analyses of raw materials and samples of silages stored for 9 months for FRS, MT-7, KJ-14 and VB.

Table 1. Protein (N*6.25), total volatile nitrogen (TVN), trichloroacetic acid soluble nitrogen (TCA-sol.N), cadaverine and tyramine in raw materials (A), in freshly prepared silages (B) and in the silages stored for 1 year (C).

		FRS	MT-3	MT-5	MT-7	KJ-5	KJ-10	KJ-14	VB[1]
Protein	A	143	141	145	139	141	143	141	152
(g kg^{-1})	B	142	141	145	142	141	141	145	153
	C	149	149	148	149	150	148	147	152
TVN	A	1.3	3.6	6.8	9.1	4.5	5.7	10.3	1.1
(% of	B	0.6	0.9	3.9	7.3	1.0	5.1	9.0	1.0
tot. N)	C	2.7	2.9	5.3	9.4	3.0	7.3	11.3	1.4
TCA sol.N	A	18	22	28	35	23	30	39	19
(% of	B	30	31	35	37	35	34	37	19
tot. N)	C	92	90	87	86	89	89	89	35
Cadaverine	A	-	-	7.9	13.4	-	8.6	15.7	-
(gkg^{-1}	B	-	-	-	16.3	-	5.5	14.1	-
prot)	C	-	-	2.2	11.8	-	4.4	13.3	-
Tyramine	A	-	-	2.6	5.8	-	2.9	5.8	-
(gkg^{-1}	B	-	-	1.2	5.1	-	1.6	5.2	-
prot)	C	-	-	1.2	4.5	-	1.9	6.3	-

FRS[1]=raw thawed capelin. VB=cooked capelin. MT-X=stored for X days at 10-12°C. KJ-X=stored for X days at 2°C.

Table 3 shows the mean PER values (n=5) for all the silages at each storage time. The mean values are also expressed relative to the PER of the silage prepared from cooked capelin (VB). Analysis of variance of all the data (n=200) showed for the PER values significant differences between silages ($p<0.001$) and storage time ($p<0.001$). There was also a weak interaction between silages and storage times ($p<0.05$), probably because the PER of all the silages from uncooked raw material decreased with time of storage whereas the value for VB remained high throughout. There was little difference between silages prepared from raw materials of different prestorage times, that is the time the capelin was left at 10-12°C and 2°C before ensiling. During the first three months of silage storing only the silage from capelin prestored for 14 days at 2°C (KJ-14) showed relative PER values below 90% of the value for VB. At six months the silages from capelin prestored at 10-12°C for 3, 5 and 7 days (i.e. MT-3, MT-5 & MT-7) still showed relative values about 90% wheras those from capelin prestored 5, 10 and 14 days at 2°C (i.e. KJ-5, KJ-10 & KJ-14) showed relative values lower than 90%. At 12 months all silages showed relative values of 80% or lower, and there was no clear-cut relation to the prestorage conditions. In the silages MT-5, MT-7, KJ-10 and

Table 2. Amino acid (mg g^{-1} protein) composition of raw materials (R) and the corresponding silages (E) stored for 9 months at 10-12°C.

	FRS		MT-7		KJ-14		VB	
	R	E	R	E	R	E	R	E
Asp	81	82	76	71	69	68	80	82
Thr	38	41	41	41	36	36	38	39
Ser	40	39	38	25	33	33	40	40
Glu	120	116	125	102	113	95	117	116
Gly	49	48	60	51	52	48	48	47
Ala	53	50	63	52	58	49	53	49
Val	43	46	47	46	44	45	44	46
Met	30	25	35	30	29	27	27	-
Ile	37	39	41	40	38	37	37	37
Leu	71	68	74	72	72	66	72	68
Tyr	30	27	27	24	26	26	32	25
Phe	34	34	36	35	33	32	35	36
Lys	72	74	62	60	57	57	73	73
His	19	18	19	18	19	17	19	17
Arg	55	60	49	62	43	58	53	55
Hyp	5	4	7	5	3	5	5	3
Pro	36	39	41	37	35	32	32	40

FRS = raw thawed capelin
MT-7 = capelin stored for 7 days at 10-12°C
KJ-14 = capelin stored for 14 days at 2°C
VB = cooked capelin

KJ-14 biogenic amines were found, in MT-5 and KJ-10 the amounts were negligible but in MT-7 and KJ-14 the amounts corresponded to more than 20% of the lysine and tyrosine originally present in the raw material (Table 1).

In Table 4 mean values (n=5) for apparent protein digestibility are given. Analysis of variance of all the data (n=200) showed no significant differences. However, when the means were considered (n=40), the mean for samples stored for one year was significantly lower than the other means ($p<0.001$). A similar analysis of the mean feed intake data (n=40) showed significantly lower mean feed intake in silages stored for one year than the means for the other storage times ($p<0.001$). When means were taken over silages the mean intake for VB was higher ($p<0.01$) and that for KJ-14 was lower ($p<0.01$) than the means for the other silages (Table 5).

The nitrogen balance experiment with the freshly prepared silages FRS, MT-5, KJ-5 and VB showed slightly better nitrogen balance (Bal%) and net protein utilization (NPU) for FRS than for the others, included VB. These silages were also tested for protein digestibility in mink at the Herring Oil and

Table 3. Mean PER values (n=5) in each experiment and PER values relative to the PER values of VB.

Silages/Time	1 week	6 weeks	3 months	6 months	12 months
FRS[1]	2.88	3.08	2.94	2.64	2.26
	.914	.925	.980	.923	.743
MT-3	3.02	3.03	2.75	2.65	2.12
	.959	.910	.916	.926	.679
MT-5	3.02	3.14	2.86	2.57	2.36
	.959	.943	.953	.899	.776
MT-7	2.98	3.04	2.83	2.59	2.46
	.946	.913	.943	.905	.809
KJ-5	3.15	3.19	2.76	2.40	2.21
	1.000	.958	.920	.839	.727
KJ-10	3.02	3.03	2.83	2.46	2.34
	.959	.910	.943	.860	.770
KJ-14	2.78	2.84	2.40	2.25	2.09
	.882	.853	.800	.787	.687
VB	3.15	3.33	3.00	2.86	3.04
	1.000	1.000	1.000	1.000	1.000

Mean±pooled SEM (FRS-VB) 2.748±0.
Mean±pooled SEM (FRS-KJ14) 2.713±0.
Mean±pooled SEM (FRS-KJ14) 0.880±0.00668

[1]See Table 1.

Table 4. Apparent digestibility (AD, %) of protein obtained in experiments with the different silages after 1 and 6 weeks and after 3, 6 and 12 months of storage at 10-12°C.

Silages/Time	1 week	6 weeks	3 months	6 months	12 months
FRS[1]	83.1	88.1	79.7	79.9	78.4
MT-3	83.0	86.0	78.4	79.0	78.6
MT-5	83.3	83.3	78.2	80.4	75.9
MT-7	83.5	83.8	79.5	80.7	77.1
KJ-5	83.0	83.6	78.9	77.4	76.2
KJ-10	81.3	83.9	81.6	81.0	74.7
KJ-14	82.9	82.2	76.7	79.7	71.8
VB	80.9	85.1	75.9	78.5	75.3

Means±pooled SEM (FRS-VB) = 80.26±0.273
[1]See Table.1

Meal Research Institute (SSF) without showing differences between silages. The results are included in Table 6 together with the results of the nitrogen balance experiment with rats.

Table 5. Feed intakes (g rat^{-1} day^{-1}) during the growth experiment of 24 days fed the different silages stored at 10-12°C.

Silages/Time	1 week	6 weeks	3 months	6 months	12 months
FRS[1]	15.8	14.4	15.7	17.1	14.2
MT-3	16.0	15.8	15.2	17.6	11.2
MT-5	16.0	15.6	15.6	16.5	14.0
MT-7	15.3	15.0	14.2	15.5	13.8
KJ-5	16.6	15.9	16.0	15.5	14.0
KJ-10	15.9	15.2	15.2	15.5	12.4
KJ-14	14.3	14.9	12.9	14.3	11.9
VB	15.8	16.0	15.5	16.0	15.1

Mean±pooled SEM=15.081±0.147
[1]See Table 1.

Table 6. Apparent digestibility (AD) and true digestibility (TD) of protein, nitrogen balance (Bal%) and net protein utilization (NPU) in the nitrogen balance experiment with rats fed newly produced silages from the raw material treated differently prior to ensiling. True digestibilities obtained in an experiment with mink fed the same silages is also included.

Silages	AD	TD	Bal%	NPU%	TD_{mink}
FRS[1]	85.8	98.5	62.8	82.4	94.3
MT-5	85.2	97.8	57.4	77.3	92.5
KJ-5	84.3	96.9	57.0	77.6	93.3
VB	84.0	96.7	56.6	76.7	92.7

1 See Table 1.

DISCUSSION

The aim of the present study was to evaluate whether any of the chemical parameters measured by Haaland *et al.* (1990) in the 8 silages could be used to predict the results of the PER experiments and of the nitrogen balance experiment. In fish meal produced for use in feeds for fur animals and farmed fish, it is required that TVN of the raw material does not exceed 40mg $100g^{-1}$ (LT-meal, low temperature dried meal) and 90mg $100g^{-1}$ (NSM-meal, NorSeaMink-meal). This corresponds to about 1.8 and 4.0% TVN of total N. Further it is required that cadaverine does not exceed 1g and 1.8g per

kilo dry matter for the two fish meal types, respectively. This correspond to decarboxylation of maximum about 1.7% and about 3% of the lysine originally present in the raw material. All the raw materials except those for FRS and VB showed TVN values higher than 1.8% of total N (LT-meal requirement); MT-3, FRS and VB had TVN values less than 4% of total N (NSM-meal requirement). In the freshly prepared silages (Table 1), FRS, MT-3, KJ-5 and VB passed the LT-requirement with MT-5 on the borderline for the NSM-requirement, but only the FRS-silage showed slightly better values than the others. Cadaverine was detected in the raw materials for MT-5, MT-7, KJ-10 and KJ-14 and in freshly prepared silages MT-7, KJ-10 and KJ-14. For the three latter the amounts corresponded to about 30%, about 10% and about 25%, respectively, of the lysine originally present. There is a rough agreement with theamounts of lysine found in the raw materials and the silages MT-7 and KJ-14 as compared to the amounts found in the raw material and silages FRS and VB (Table 2). We had some difficulties with the cadaverine determination and advocate that the tyramine content be used as an alternative to cadaverine. Thus both the TVN-values and the cadaverine values indicate that the raw materials for the silages MT-7, KJ-10 and KJ-14 were inferior to the requirements but only the silage KJ-14 showed consistently lower PER values than the other silages when they were freshly prepared and up to 6 months storage, after storage for one year all silages had low PER values. We have no explanation for this, but different degrees of autolysis might be suggested to account for the differences. However TCA-soluble nitrogen were not different between silages during the storage period of one year. As mentioned earlier lower protein utilization in silages stored for long periods were often observed (Strøm and Eggum, 1981; Espe *et al.* 1989, 1990, 1992a; Haaland and Njaa 1990). On the other hand diets in which silage protein accounted for parts of the fish protein were utilized equally to diets with only fish meal protein by chickens (Espe *et al.*, 1992b) and by Atlantic salmon (Espe *et al.*, 1992c). Also big salmon fed diets containing about 20% of silage protein grew as well as salmon on a commercial dry diet (Lie *et al.*, 1988).

The effect of the quality of the raw material on the biological quality of fish silage has been little studied. The experiment reported by Haaland and Njaa (1990) on silages prepared from capelin prestored for 1, 3 and 5 days at ambient temperature showed that storage for 1 and 3 days gave similar net protein utilizations of the silages, but the silage prepared from the raw material stored for 5 days gave significantly lower value. This was the fact with newly produced silages, but when the silages had been stored for 3 months there were no differences between them, all showing low net protein utilization.

CONCLUSION

The aim of the present experiment was to evaluate whether chemical criteria relating to raw materials used for preparation of fish silages could predict the outcome of biological protein evaluation tests with the silages. The results showed that even when the raw materials contained relatively high amounts of TVN (total volatile nitrogen) and amines (cadaverine and tyramine) the protein utilization as measured by PER (protein efficiency ratio) was only affected to a small extent. This is probably due to that the two most vulnerable amino acids (lysine and tyrosine) were in excess of the rats requirements. The most pronounced effect on the utilization of protein was from the time of storing the silages before use. The silages from uncooked raw material all showed reduced protein utilization when they had been stored for one year. We have no explanation of this fact, but we assume that the degree of autolysis may be of importance as the silage from cooked raw material did retain its protein quality. The digestibility of silage protein was not different between silages as measured in rats and mink.

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CONTENTS OF B-VITAMINS IN FISH

By

EINAR LIED
Institute of Nutrition, Directorate of Fisheries
N-5024 Bergen, Norway

ABSTRACT

To obtain information on the nutritive value of raw materials to be used in feed for fur animals and in fish farming samples of 14 species of whole fish, 5 samples of krill and red feed as well as 3 samples of fish offal were analysed for their contents of the B-vitamins thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, vitamin B_{12} and biotin. All vitamins were determined by microbiological methods. The results are reported in tables as mg/kg dry matter.

INTRODUCTION

A project on the analysis of nutrients in Norwegian fish species and fish products was sponsored by the Norwegian Council for Fisheries Research for the years 1973-1975. Results were published at intervals in «Fiskets Gang» (UTNE, 1976). The amino acid analyses were compiled and published by NJAA and UTNE (1982), the fatty acid composition of the fish lipids by LAMBERTSEN (1978) and data on mineral contents by JULSHAMN *et al.* (1978).

To complete this series of papers reporting on the nutrient content of fish relevant as raw materials or ingredients in the production of feed for fur animals and in fish farming this communication reports on the contents of B-vitamins. Most samples consisted of whole fish; however, samples of krill and red feed were included as well as a few samples of fish offal.

METHODS

In most cases analyses were performed in 3 samples of each species. However, in a few cases only 2 samples were obtained. The samples were brought to the insitute fresh, frozen or in ice. They were in good condition as indicated by their low contents of trimethylamine and high values of trimethylamine oxide (Njaa and Utne, 1982). The samples were homogenized by several passes

through a meat grinder at low temperatures and representative portions were frozen and used for analysis.

Analyses were carried out in wet samples. All vitamins were determined by microbiological growth assays. Thiamine (vitamin B_1) was determined with *Lactobacillus viridescens* (ATCC 12706) according to DEIBEL et al. (1957), riboflavin (vitamin B_2) was determined with *Leuconostoc mesenteroides* (ATCC 10100) according to BARTON-WRIGHT (1963), niacin and pantothenic acid were determined with *Lactobacillus plantarum* (ATCC 8014) according to AOAC Methods (1980), biotin was determined with *Lactobacillus plantarum* according to Pharmacopoea Nordica (1960), vitamin B_{12} with *Leuconostoc mesenteroides* (ATCC 4797) using an growth assay medium from Ferrosan, Denmark, and pyridoxine (vitamin B_6) with *Saccharomyces carlsbergensis* (ATCC 9080) according to AOAC (1980).

RESULTS AND DISCUSSION

The contents of B-vitamins in the analysed samples are shown in Table 1. The analyses were performed in wet samples. The results are, however, recalculated based on the dry matter values reported by UTNE (1976) and reported as mg per kg dry weight.

The content of thiamine in whole fish varied from 0.3 to 20.3 mg/kg. The majority of the fish samples were low in thiamine. Several of these fish species contain the enzyme thiaminase. This enzyme catalyse a reaction, which splits the thiamine molecule into a thiazole and pyrimidine moiety and thereby destroys the biological activity of the vitamine. Krill and red feed shows thiamine values of 14 to 21 mg/kg, which are comparable to those found in saithe, Norway pout and blue whiting, fish species known not to possess thiaminase. The content of this enzyme should be taken into account when using fish species as capelin, sprat and herring as components of fish feeds in order to avoid a deficiency of thiamine.

With the exception of offal from spur-dog (heads) and prawn, which were found to be low in riboflavin, the contents of this vitamine varied from about 5 to about 13 mg/kg, showing an average riboflavin value of 8.8 mg/kg. Niacin varied from 47 to 218 mg/kg, averaging 111 mg/kg. The highest values were found in the fish species mackerel, sandeel, local stock herring and sprat, while prawn waste was very low in its content of this vitamin. The content of pantothenic acid showed less variation between samples, the highest values were found in samples of krill, red feed, North sea stock herring, capelin, sandeel and in the male lumpfish. The average content of this vitamin in the analysed samples was 26 mg/kg, the highest value was 45 mg/kg in krill and the lowest value 16 mg/kg in whole saithe. A lower value was found in boiled krill. In general, boiling extracted a significant amount of all vitamins from

Table 1. The content of B-vitamins in fish. The values are given as mg per kg dry matter. n.a. = not analysed.

Species	Dry weight %	Thiamine	Riboflavin	Niacin	Pant. acid	Pyridoxine	B-12	Biotin
			Milligram per kg. dry weight					
Great silver smelt .... *Argentina silus*	25.0	6.3	10.2	96.0	22.8	0.12	0.24	7.24
Saithe, whole ..... *Pallacius virens*	24.5	12.9	9.9	117.1	16.3	6.33	0.16	0.26
Saithe, gutted ..... *Pollacius virens*	21.7	15.9	8.6	123.5	19.4	9.03	0.10	0.58
Norway pout ..... *Boreogadus esmarkii*	27.6	20.3	8.8	74.3	20.2	3.80	0.21	0.34
Blue whiting ..... *Micromesistitius poutassou*	24.7	19.9	13.4	84.2	23.8	6.19	0.34	0.29
Sprat ..... Sprattus sprattus	29.5	2.7	5.0	159.3	20.3	5.56	0.25	0.23
Müllers pearlsides ..... *Maurolicus muelleri*	30.0	0.8	13.4	107.3	27.5	2.85	0.56	1.98
Herring, local fjord stock ..... *Clupea harengus*	36.6	0.6	4.5	158.5	23.2	7.38	0.21	0.23
Herring, North sea stock ..... *Clupea harengus*	35.2	0.3	6.5	123.3	34.7	0.80	0.24	0.26
Capelin ..... *Mallotus villosus*	25.6	0.3	10.7	87.1	32.7	0.34	0.44	3.83
Mackerel ..... *Scomber scombrus*	36.3	6.6	10.5	218.2	26.4	15,98	0.28	0.28
Sandeel ..... *Ammodytes tobianus*	27.1	8.8	10.0	169.0	36.3	10.00	0.28	0.74
Lumpfish, male ..... *Cyclopterus lumpus*	13.5	4.1	7.4	88.9	34.8	4.00	0.41	0.30
Lumpfish, female .... *Cyclopterus lumpus*	10.1	3.3	7.3	94.1	25.7	5.45	0.22	0.27
Ballan wrasse ..... *Labrus bergylta*	27.5	3,3	7.1	66.5	22.4	4.11	0.20	0.20
Krill, Antarctic, fresh *Euphausia superba*	19.4	21.4	10.3	122.7	45.4	7.68	1.03	n.a.
Krill, Arctic, fresh .... *Meganyctiphanes norvegica*	19.3	20.7	11.3	143.5	44.9	9.22	0.68	0.31
Krill, Arctic, boiled *Meganyctiphanes norvegica*	20.4	7.0	5.6	90.2	13.6	4.07	0.29	0.34
Red feed, calanus, fresh ..... *Calanus finmarchicus*	19.4	17.4	9.3	120.1	30.9	5.67	0.75	0.80

Species	Dry weight %	Thia-mine	Ribo-flavin	Niacin	Pant. acid	Pyri-doxine	B-12	Biotin
			Milligram per kg. dry weight					
Red feed, calanus, boiled ..... *Calanus finmarchicus*	21.3	13.9	7.1	80.8	19.6	3.61	0.83	0.72
Cod heads ..... *Gadus morhua*	21.1	20.1	8.2	47.3	23.1	5.36	0.08	0.09
Spur-dog, heads ..... *Squalus acanthias*	21.4	9.3	2.7	87.9	18.5	9.44	0.11	0.31
Prawn waste ..... *Pandalus borealis*	18.8	2,6	2,1	8.3	n.a	1.86	0.16	0.19

the samples with the exeption of biotin in krill, and biotin and vitamin B_{12} in the red feed.

The average content of pyridoxine in all samples was 5.9 mg/kg. The contents of this vitamin varied to a greater extent between samples than that of riboflavin, niacin and pantothenic acid. The content of pyridoxine in great silver smelt was extremely low (0.12 mg/kg) while the content of mackerel was very high (about 16 mg/kg). The average contents of vitamin B_{12} and biotin were 0.35 and 0.41 mg/kg, respectively. In many cases the contents in the samples of these two vitamins parallelled each other (e.g. great silver smelt, blue whiting, sprat, herring, mackerel, ballan wrasse). High values of vitamin B_{12} were found in Müllers pearlsides, krill and red feed. The lower value of this vitamin in gutted saithe as compared with whole saithe indicates that the liver containes a significant amount of vitamin B_{12}. A very high content of biotin was found in Müllers pearlsides, while sandeel, red feed and gutted saithe showed values higher than the average value of 0.41 mg/kg for all samples.

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