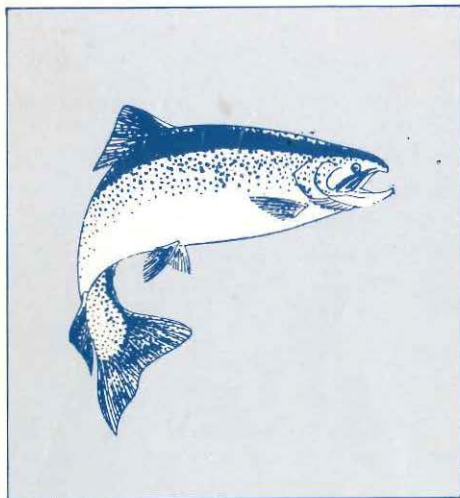


International Symposium on Feeding and Nutrition in Fish

Bergen, 23–27 August 1987



ABSTRACTS

10ⁿ-10¹⁵ 24/8/87

INFLUENCE OF FEEDING ON PROTEIN METABOLISM IN ATLANTIC
SALMON (Salmo salar).

B. FAUCONNEAU & C. BIELLE

Fish Nutrition Laboratory, I.N.R.A.,
Station d'Hydrobiologie,
64310 Saint Pée-sur-Nivelle, France.

Two experiments were conducted to study the post-prandial changes in protein synthesis rate of different tissues in juvenile Atlantic salmon using an i.v. injection of a large dose (1.5 μ moles/g) of L-[2,3-³H]-leucine.

In the first experiment the salmon were regularly fed a commercial diet in two meal per day (mean body weight: 36.8g). Immediate feeding (2 h after the morning meal compared to overnight fast) did not significantly affect the protein synthesis rate of the liver, gill, and muscle.

In the second experiment, the changes in protein synthesis rate of the muscle and scales were followed over a period of 18 h after a meal, in salmon (mean body weight 43.4 g) starved since one week. The rate of protein synthesis in muscle increased until 18 h after feeding. No significant changes were observed in the protein synthesis rate of the scales.

10¹⁵-10³⁰ 24/87
NUTRITIONAL EFFECTS ON MUSCLE PROTEIN SYNTHESIS IN COD
(GADUS MORHUA)

Einar Lied

Institute of Nutrition, Directorate of Fisheries
P.O. Box 4285
N-5028 Bergen
NORWAY

Cod (Gadus morhua) was starved for 8 days and refed ad lib. In the first experiment amino acid incorporating activity was determined by ribosomes isolated from white trunk muscle sampled 4, 8 and 12 hr of refeeding. The ratios in area between poly- and monoribosomes were determined. Amino acid incorporating activity was reduced to 12-18% that of normal fed fish after 8 days of starvation. Activity was restored close to the level of normal fed fish after 12 hr of refeeding. Polyribosomal aggregates increased again within 4 hr of refeeding. The content of ribosomal RNA but not that of DNA per g wet weight decreased during the 8 days of starvation and regained the normal fed fish level within 8 hrs of refeeding.

In the second experiment ¹⁴C-phenylalanine was included in the diet. Samples of white trunk muscle were collected 4, 8, 12, 24, 48, 72 and 96 hr of refeeding and radioactivity measured in homogenates of whole muscle, in the TCA precipitate and in the TCA supernatant. The incorporation of isotope in the TCA precipitate increased linearly with time during 4 to 72 hr of refeeding.

10¹⁰-11⁰⁵ ✓

SHORT-TERM RESPONSES OF PROTEIN SYNTHESIS TO RE-FEEDING IN RAINBOW TROUT.

D.N. McMillan and D.F. Houlihan.

3H-phe

Department of Zoology,
University of Aberdeen,
Aberdeen, Scotland.
AB9 2TN.

The fractional rate of protein synthesis (ks, %/day) was measured in various tissues of rainbow trout using the flooding dose technique (Garlick et al., 1980). This was shown to be a reliable method for use in fish (Houlihan et al., 1986; McMillan, 1987). Fish were either fasted for 6 days, or fasted for 6 days and then re-fed in one meal. In the re-fed fish, protein synthesis was measured 3 hours, 6 hours and 12 hours later. A continually fed group was included as a control. The tissue RNA:protein ratio was determined so that protein synthesis could be expressed per unit of RNA (giving an indication of the ribosomal activity - Millward et al., 1975). This 6 day period of fasting had little effect upon the tissue RNA:protein ratio as only the stomach and gill had significantly lower levels than the continually fed fish. The liver was the only tissue which did not show a fall in the fractional rate of protein synthesis with fasting. The fractional rate of protein synthesis in the tissues of the fasted fish showed a strong linear correlation with the RNA:protein ratio. The nature and rapidity of the response of protein synthesis to re-feeding was tissue-specific. Within 3 hours of feeding there was a marked stimulation of protein synthesis in the gill, stomach, intestine and liver. This increase in the liver was transient as protein synthesis returned to the levels in the fasted and fed fish. Thus, the liver exhibited a 'spike' in protein synthesis in response to re-feeding. (The nature and subcellular localisation of the proteins being synthesised during this 'spike' is currently under investigation). The white muscle did not exhibit increased protein synthesis until 6 hours after the meal. Re-feeding had little effect on tissue RNA:protein ratio so that these increases in tissue protein synthesis were brought about through changes in the rate of synthesis per unit RNA. That is, the re-feeding stimulus was affecting tissue protein synthesis at the level of translation and not by altering the tissue RNA content.

- References: Garlick, P.J., M.A. McNurlan & V.R. Preedy (1980).
Biochem. J. 192: 719-723.
Houlihan, D.F., D.N. McMillan & P. Laurent (1986).
Physiol. Zool. 59(4): 482-493.
McMillan, D.N. (1987). PhD Thesis, University of
Aberdeen.
Millward, D.J., P.J. Garlick, R.J.C. Stewart, D.O.
Nnanyelugo & J.C. Waterlow (1975).
Biochem. J. 150: 235-243.

MYOSIN SYNTHESIS IN WHITE TRUNK MUSCLE OF RATION FED COD (GADUS MORHUA)

von der Decken, Alexandra

The Wenner-Gren Institute for Experimental Biology,
University of Stockholm, S-106 91 Stockholm, Sweden.

It has been shown (Lied et al. 1985, *In Nutrition and Feeding in Fish*, Ed. Cowey et al., Academic Press) that in ration fed cod (*Gadus morhua*) protein synthesis decreases in epaxial muscle at ration sizes of 50% and 5% that of ad libitum feeding while it remains unchanged at a ration size of 75%. The question arises whether the contractile or the sarcoplasmic proteins are preferentially affected by the diminished food energy intake. Myosin heavy chain was chosen as being representative of contractile proteins. The protein was isolated previously and antibodies were raised in rabbits (Lied and von der Decken, *Biochem. J.* 232, 467-470, 1985).

Ribosomes were isolated from epaxial muscle. The ribosome fraction consisted in addition to the ribosomes of other proteins especially of actomyosin complexes. These additional proteins were removed by resuspension and precipitation of the ribosomes in 4% NaCl-70% ethanol. This procedure increased the RNA/protein ratio from 0.058 ± 0.005 to 0.323 ± 0.032 .

The myosin content was determined by the ELISA assay technique. In the whole ribosome fraction (not NaCl-ethanol precipitated) the content decreased between ad libitum and 75% ration size, increased significantly at 50% and decreased again at 25% ration size. The precipitated ribosomes containing myosin under synthesis was unchanged between ad libitum and 75% ration size feeding and decreased at 50% and 25% ration size. The results show that myosin synthesis decreased at low ration sizes and that myosin relative to other proteins present in the whole ribosome fraction increased at 50% but decreased at 25% ration size. The results on myosin under synthesis are in agreement with the observations by Lied et al. 1985 on a decrease in overall protein synthesis at 50% and lower ration sizes. Hybridization between messenger RNA and cloned myosin heavy chain DNA indicated a similar trend in results showing a higher content of myosin messenger RNA at ad libitum food intake than at 25% ration sizes.

Support: The Swedish Council for Forestry and Agriculture Research, Project No. 520/85 DV38 and The Swedish Natural Science Research Council, Project No. B-Bu 2520-111.

11-11
10-11
25

UTILIZATION OF DIETARY PROTEIN BY THE EEL (*Anguilla anguilla*). OPTIMUM DIETARY PROTEIN LEVELS.

M. de la Higuera, M. Garcia Gallego, A. Sanz, M.C. Hidalgo and
M.D. Suarez

Departamento de Biología Animal, Ecología y Genética
Facultad de Ciencias. Universidad de Granada.
18071 Granada
Spain

The aim of this work was to establish approximate optimum levels of protein in the diet of the eel (*Anguilla anguilla*), through growth and protein utilization studies. With that purpose, wild eels from Spanish south-west coast marshes, were size-weight selected and randomly distributed in fiber-glass tanks with a continuous aeration and fresh water supply, at a flow of 1 l/min and at a temperature of 25±1°C. After 1 month of adaptation to experimental conditions and feed replicate groups of 50 fish (40 g mean body weight/fish) were fed the experimental diets for 2 months, twice a day (at 10 and 17 h time). Diets were formulated to get protein levels of 35, 40, 45, 50 and 55%, 12% fat and varying levels of carbohydrates in order to make them theoretically isoenergetics (14.6 MJ Metabolizable Energy/Kg diet). Two protein sources were independently tested, herring and white fish meal, at the forementioned levels of protein and in duplicate groups for each source. Essential amino acid contents of both protein sources would cover requirements of japanese eel, as the requirements of the european eel have not yet been established.

Weight increase, food conversion index, protein efficiency ratio (PER) and protein productive value (PPV) were similar for diets with 45, 50 and 55% protein, for both protein sources. Nevertheless, these same parameters improved for white fish meal diets at protein levels of 35 and 40%. Absolute intake of protein for optimal growth, food conversion and nitrogen retention (PPV) corresponds to the intake of a diet with a 40% protein. Apparent digestibility of protein, using chromic oxide as indicator (1% of the diet), was higher for diets formulated with the white fish meal. Results of individual body weight increase were not homogeneous, giving extreme values of 10 and more than 120% increase in most of the experimental groups, as it has also been shown, for the japanese and european eel, in the literature. In consequence, the mean values obtained for food conversion and nitrogen retention are low. Anyhow, a certain good correlation between dietary protein levels and feed conversion rates, PER and PPV was observed.

Best results were obtained with the 40% white fish meal protein diet which would indicate that protein requirements of eel, at this growth stage, would be around this value. These preliminary results would need of a further research in order to establish protein requirements according to a possible more adequate protein/energy ratio in the diet of the european eel, at different growth stages.

This work was supported by a C.A.I.C.Y.T. grant.

11-25-1140

EFFECTS OF FEEDING RAINBOW TROUT DIETS CONTAINING WHOLE PROTEIN, PROTEIN ENZYMATIC HYDROLYSATE OR AN AMINO ACID MIXTURE ON THE METABOLISM OF LIVER AND GASTRO-INTESTINAL TRACT

M.J.WALTON, R.K.BUDDINGTON & C.B.COWEY

NERC, INSTITUTE OF MARINE BIOCHEMISTRY, ST FITTICK'S ROAD
ABERDEEN, AB1 3EH, SCOTLAND, U.K.

Diets containing substantial quantities of free amino acids are frequently used in nutritional experiments with fish. The addition of free amino acids or protein hydrolysates (and silages) to improve poor 'quality' proteins has also been utilized. However in most investigations much better growth was obtained when whole (good quality) protein was fed compared to when free amino acids were present. The current studies were performed to investigate the effect of different dietary forms of amino acids on their metabolism in rainbow trout. Duplicate tanks (42 fish/tank) of trout (mean wt. 25g) were fed one of 3 isonitrogenous and isocaloric diets of identical amino acid composition containing either casein (C), casein enzymatic hydrolysate which is a mix of short peptides (H) or an amino acid mixture (A). At the end of the feeding period (7 weeks) fish fed diets H (2.28%) and A (2.32%) had significantly (Dunnet's test) higher hepatosomatic indices (HSI) than those fed C (1.68%). This was primarily due to an accumulation of glycogen (C 122; H 268; A 260 mg/liver of 100g fish) although protein and lipid levels were also slightly higher in fish fed A or H. Possible reasons for these differences in metabolism were investigated. Activities of digestive enzymes and uptake capabilities of everted gastro-intestinal preparations were measured (see accompanying abstract by Buddington et al.). Few significant differences were found in the activities of enzymes of intermediary metabolism in liver i.e. no differences for GOT, GPT, GLDH, Histidase, Arginase, Ser-Pyr transaminase or PK; LDH, FDPase, PFK and Cit synthase were higher in fish fed A or H compared to C (all activities were expressed as U/liver of 100g fish to correct for the differences in HSI).

No differences were seen in the extra/intra cellular distribution ratios of the non-metabolizable amino acid analogue AIB in liver, kidney, white muscle, red muscle or gills at both 6 and 24 hr after feeding. Also blood samples (9 fish/diet at each period) were collected at 0,3,6,12,24 and 48 hr after feeding each of the diets. Amino acid analyses revealed greater EAA/NEAA ratios in the blood of fish fed diets H (1.04) and A (1.13) compared to C (0.65) during the absorptive phase. This was due to high (imbalanced) levels of the EAA especially the branched chain amino acids.

The results indicate that a greater proportion of amino acids from diets H and A than amino acids from diet C is directed towards non-protein synthesising pathways such as glycogen formation.

M⁴⁰ - M⁵⁰

EFFECT OF DIGESTIBLE CARBOHYDRATES ON PROTEIN/ENERGY
UTILIZATION AND ON CARBOHYDRATE METABOLISM IN RAINBOW TROUT

S.J. KAUSHIK, F. MEDALE, B. FAUCONNEAU, & D. BLANC

Fish Nutrition Laboratory, I.N.R.A.,
Station d'Hydrobiologie,
64310 Saint Pée-sur-Nivelle, France.

Groups of 150 rainbow trout (Mean body weight : 28 g) were pair-fed five different diets containing 36 % of a high quality protein source (Norseamink) and various carbohydrate sources. All diets were formulated and pair-fed in such a way as to provide identical amounts (in g/Kg Body weight) of digestible protein, digestible energy and of carbohydrates. Diet 1 containing 38 % of crude corn starch (Crude protein / fat : 36/18) served as the control. Experimental diets 2 to 5 contained different extruded carbohydrate sources : whole wheat, whole corn, wheat starch and corn starch respectively. Growth studies were performed at a water temperature of 17.5°C over 15 weeks. Postprandial nitrogen excretion rates, glycemia and respiratory quotients (R.Q) were measured in order to evaluate utilization of specific energy-yielding substrates. After an intravenous injection of labelled (3-H-) 2-deoxyglucose, plasma clearance of glucose and the effect of insulin on such glucose utilization were measured in three of the five experimental groups.

Incorporation of extruded carbohydrates increased the digestibility of dry matter of the diets. The source of carbohydrates had little effect on protein utilization while energy and fat utilization were affected. Extruded wheat starch appeared to be a better energy source than other raw materials tested. Nitrogen and energy balance studies did not show great difference between groups. Good tally was found between nitrogen and energy balance studies made after measurement of the fecal, metabolic or respiratory losses and retention values after carcass analyses. Non-protein substrate utilization was different with the control diet (18 fat / 38 crude starch) than with the other diets containing extruded carbohydrate sources. The hypoglycemic effect of insulin was greater in crude-starch fed trout than in fish of other groups. Fish adapted to long-term ingestion of digestible carbohydrates showed a slightly but not significantly poorer utilization of glucose. Glucose utilization in rainbow trout appears to show little or no metabolic adaptation even after long-term ingestion of digestible carbohydrates.

AN EVALUATION OF THE ISOLATED, IN VITRO, NON-MYOTOMAL MUSCLE
PREPARATION FOR THE STUDY OF FACTORS AFFECTING PROTEIN SYNTHESIS
IN FISH

WRIGLEY, J.O.L. & ASH, R.

School of Biomedical Sciences,
University of Bradford,
Richmond Road,
Bradford BD7 1DP,
West Yorkshire, U.K.

Muscle tissue is, on a dry weight basis, largely protein and constitutes the single largest component of fish body mass and hence saleable/edible product. For this reason it is apparent that the efficiency with which ingested nutrients are converted into protein (muscle mass) must be of considerable significance to the fish culturist. Net changes in body protein content may arise as a result of metabolic adaptations which may affect either, or both, of the processes of protein synthesis or degradation. Factors affecting these processes are many and although *in vivo* methods can provide a means for assessment of their overall, integrated action such methods cannot generally provide the opportunity to systematically 'dissect out' and assess the response to independent factors such as the availability of specific nutrient(s) (e.g. individual amino acids or profiles thereof), or hormones etc. In contrast, *in vitro* systems do provide the opportunity to directly manipulate conditions under which an isolated preparation is maintained. Such flexibility and the ability to monitor metabolic changes occasioned by selected, controlled alterations to the incubation/perfusion medium can, however, only be obtained by forfeiture of various integrated physiological associations inherent in the normal functioning of the whole animal. The degree of such forfeiture will necessarily have implications with respect to the interpretation of results obtained from *in vitro* systems and their subsequent extrapolation to the *in vivo* situation. Nevertheless, by careful design of experimental systems and due regard to interpretive limitations, appropriate *in vitro* preparations can provide valuable predictive information relating to the significance of various factors to the metabolism of specific tissues.

This communication will describe the use of isolated, intact supra-, and infra-carinalis muscles of the carp for the study of factors affecting protein synthesis in the fish. The basic methodology employed was that described by Frayn & Maycock (1979) for the study of mouse muscle metabolism adapted, where appropriate, for application to the fish. Protein synthetic rates were monitored by specific reference to [³H]-tyrosine incorporation as described by Frayn & Maycock (1979). The communication will provide a critical evaluation of this technique as regards its applicability to fish muscle preparations and include both presentation and discussion of original data obtained from preparations in which perturbations were made with respect to the standard incubation medium (control system) in terms of the availability of amino acids and specific hormones.

Frayn, K.N. & Maycock, P.F. (1979). Biochem.J., 184, 323-330.

NUTRITIONAL CONTROL OF PROTEIN SYNTHESIS IN COD.

D F HOULIHAN and S J HALL *

ZOOLOGY DEPARTMENT, ABERDEEN UNIVERSITY, ABERDEEN, SCOTLAND, UK.

* MARINE LABORATORY, VICTORIA ROAD, ABERDEEN, SCOTLAND, UK.

Cod were maintained on variable rations and the relationships between protein input and protein deposition determined. Measurement of the RNA to protein ratios in tissues and the whole animal revealed that there was a linear relationship between protein input and tissue RNA. RNA to protein ratios were in turn linearly related to rates of protein synthesis in the whole body and individual tissues. Protein deposition (growth) occurred at approximately one third of the rate of protein synthesis in the whole animal and this proportion increased as the ration increased. The relationships between protein deposition and post prandial oxygen consumption will be discussed.

LONG TERM EFFECT OF A HIGH PROTEIN DIET ON THE PRIMARY LIVER AND KIDNEY METABOLISM IN RAINBOW TROUT *Salmo gairdneri*

J.A. Lupiáñez*, M.J. Sánchez-Lozano, L. García-Rejón and M. de la Higuera.

*Departamento de Bioquímica y Biología Molecular y Departamento de Biología Animal, Ecología y Genética. Universidad de Granada. 18001 Granada. Spain.

The effects of a high protein diet on the kinetic of some of the more relevant enzymes of carbohydrates and nitrogen metabolism in the liver and kidney of Rainbow trout, were studied. Unlike fats, protein can not be stored as such, when they are ingested in certain large proportion; instead, they are metabolized in some fashion. For that, the amino acids exceeding the quantities required for protein synthesis, after being transformed into oxoacids can be either oxidized through the tricarboxylic acids cycle or utilized in the synthesis of carbohydrates and lipids. Thus, the aim of this work was to study the metabolic changes that occur when trouts are fed with a high protein diet.

Rainbow trout, obtained from a local fish farm, an with an individual mean boby weight of 150g, were maintained in 350l fiber-glass tanks, with a continuous aeration and fresh water supply at a flow of 1.5l/min and at a temperature of $15 \pm 0.5^\circ\text{C}$. Photoperiod was maintained at 12h. light/12 h./dark; after 2 weeks of adaptation to the laboratory conditions, trouts were fed with the experimental diets, ad libitum, twice a day (10 and 17h. time), during 4 weeks before measuring enzyme activities and kinetics. The general composition of the diets was 60% protein (70% herring meal/30% casein), 8% fat and 0% carbohydrates for the high protein diet and 45% protein (70% herring meal/30% casein), 8% fat and 20% carbohydrates (cooked starch) for the control diet. Diet influence on fructose 1,6-bisphosphatase (FBPase), phosphofructokinase (PFK), pyruvatekinase (PK), glucose 6-phosphate dehydrogenase (G6PDH), glutamate dehydrogenase (GDH) and alanine aminotransferase (AAT) activities were measured in a wide range of substrate concentrations.

Results pointed out interesting adaptative responses of trout liver and kidney metabolism after being fed on a high protein diet. Under these nutritional conditions the activities of FBPase, PFK and PK in both liver and kidney were higher than controls with different behaviour in the interactions of these enzymes with their substrates. On the other hand, the specific activity of liver G6PDH was also higher, while no change in the kidney activity was detected. With regard to the kinetic behaviour of the enzymes involved in the nitrogen metabolism, maximum velocities of liver GDH and AAT and kidney GDH were higher after feeding the high protein diet, without apparent changes in their K_m values. The metabolic and molecular changes involved in this nutritional situation will be discussed.

This work has been supported by a grant nº 1/2-85 (Junta de Andalucía)

CAN THE TROUT ADAPT TO THE FEEDING OF FREE AMINO ACIDS AND PROTEIN HYDROLYZATES? DIGESTIVE RESPONSES.

Randal K. Buddington, Michael J. Walton, and Colin B. Cowey

Address all correspondence to RKB: Department of Physiology, UCLA Medical Center, Los Angeles, CA 90024 USA

Our observations and those from other studies of the plasma composition of trout suggest that the lower utilization of free amino acids and protein hydrolyzates relative to protein may be related to the temporal patterns of amino acid availability, not the absolute quantities of amino acids available. Since the appearance of amino acids into the plasma is dependent on digestion, we investigated the potential adaptation of the intestinal hydrolytic and absorptive processes and resulting plasma constituents of rainbow trout in response to long-term feeding (10 weeks) of diets formulated with casein (diet C), a casein hydrolyzate (diet H), and an amino acid mixture similar in composition to casein (diet AA). Growth rates were similar among the three dietary treatments. After the prolonged feeding period the peak levels for the sum of all amino acids in the plasma were similar among the treatments. However, the ratio of the essential amino acids (ESS) relative to the non-essential amino acids (NESS) were higher in the H and AA groups (0.95, 1.05 and 0.59, for H, AA and C, respectively). The elevated ratio was due principally to the branched chain amino acids (in same order: BCAA/NESS=.73, .81, .38; non-BCAA/NESS=.23, .21, .22). The rates of digestion of the three diets were similar based on the appearance of xylose in the plasma. Higher intestinal hydrolytic capacities, indicated by increased tryptic, maltase and dipeptidase activities, resulted from the feeding of the H diet relative to the C diet. The AA diet also elicited an increase in the concentrations of these enzymes, though not as much as the H feed. Since the caeca contribute over 70% of the total intestinal uptake capacity of the trout (Buddington et al., 1987), we examined the potential adaptation of the absorptive processes by measuring the in vitro caecal absorption of 7 amino acids (ALA, ASP, LEU, LYS, MET, PHE, PRO) and glucose. We also determined the uptake of LEU, MET and PRO throughout the post-gastric gut. Relative to the C group, the H and AA diets enhanced the caecal uptake per mg tissue for glucose by 186 and 457%, but resulted in lower MET absorption (32% decrease for both). Uptake rates for the other amino acids were not affected by nitrogen source. Despite lower caecal uptake per mg for MET in the caeca, there were no interdiet differences in intestinal uptake capacities (nm/min-gm body weight) for MET as well as for LEU and PRO. Thus, the digestive data indicate the trout does possess some capacity to adapt its digestive processes in response to the feeding of amino acid mixtures and protein hydrolyzates. However, these responses are not sufficient to compensate for the imbalances in the ratios of ESS to NESS in the plasma, especially for the BCAA, when hydrolyzates or free amino acids are fed. These findings provide some of the basic information explaining the limited success of protein hydrolyzates as feed additives and the generally inefficient utilization of amino acids as supplements for lower quality proteins in the diets of trout, and possibly other fish.

CONTENT OF - AND TURN OVER OF - TRYPSIN AND TRYPSINOGEN
IN LARVAL HERRING DIGESTING COPEPODS

Benedikte Hedegaard Pedersen

Danish Institute for Fisheries and Marine Research
Charlottenlund Castle
DK-2920 Charlottenlund
Denmark

The content of the protease trypsin and its pancreatic proform trypsinogen was quantified by RIA in several stocks of herring larvae during the first weeks of life. Irrespective of copepod density, the content of trypsinogen fluctuated in a three-phased pattern. Digestive capability, expressed as pancreatic proform content, was low 1 week after yolk resorption relative to earlier and later life periods. Enzyme-substrate ratios in the intestine of first feeding herring larvae were extremely high - approximately 1:4. Therefore, larval ability to retain trypsin already secreted into the intestine was investigated. Retention experiments with two different herring stocks showed, that larvae apparently were capable of retaining secreted enzyme for 1 to 2 days following digestion of a single meal. Stimuli of pancreatic secretion and possible retention mechanisms for trypsin are discussed.

PLASMA INSULIN LEVELS RELATED TO GROWTH IN RAINBOW
TROUT (SALMO GAIRDNERI)

Anne Sundby, Anne Blom & Knut Eliassen

Norwegian College of Veterinary Medicine
Department of Physiology
P.O.Box 8146 Dep.
0033 Oslo 1, Norway

The relationship between growth and plasma insulin levels in 2½ year old rainbow trout was studied using a homologous radioimmunoassay. The insulin levels in fish from fast growing families were significantly higher ($p < 0.01$) than in fish from slow growing families.

When fish were selected only with respect to size (> 5.8 kg and < 1.9 kg), the difference in plasma insulin levels was even larger ($p < 0.001$). The correlation coefficient between plasma insulin level and weight in 248 rainbow trout was found to be 0.54 ($p < 0.001$).

PROTEIN AND ENERGY SOURCES FOR EUROPEAN EEL (ANGUILLA
ANGUILLA L.) DIET

Gad Degani

MIGAL - Galilee Technological Center, Kiryat Shmona,
10-200 Israel

The influence of the different protein, oil and carbohydrate sources and levels in a purified diet on the growth of the European eel (Anguilla anguilla) were examined. European eels utilize fish meal and poultry meal as a protein source and their effect on growth is significantly more than that of soya bean meal. The optimum level of protein in the eel diet is affected by the size of the fish and varied between 40-50%.

Eels fed on animal lipids (fish or poultry) grew faster than those fed on plant lipids (soya bean).

Eels can utilize various carbohydrate sources. Mono sugar was utilized better than double sugar or poly sugar.

The optimum protein/energy ratio is a very important parameter in eel diet.

AVAILABLE PROTEIN SOURCES FOR MICROPARTICULATE DIETS
OF FISH LARVAE

Akio Kanazawa, Shin-ichi Teshima and Shunsuke Koshio

Faculty of Fisheries, Kagoshima University
4-50-20 Shimoarata, Kagoshima, Japan

The present study deals with the available protein sources for larval fish fed artificial microparticulate diets. Amino acid compositions of body protein of larval fish and feed stuffs were determined by using a high performance liquid chromatography, and then various diets were formulated to have the essential amino acid patterns approximating to that of fish body protein with several protein sources. Dietary values of microparticulate diets were compared with a control diet (live food) in terms of growth and survival rates. The fish larvae used were Ayu fish, flounder and red sea bream. The larval fish fed some microparticulate diets showed good growth and high survival rates almost equal to those receiving the live food when the amino acid patterns of diets were simulated to that of the body protein of larval fish. Also, it was suggested that some plant proteins are utilized by larval fish when they are formulated to make up diets with the amino acid profile simulating that of fish body proteins.

PARTIAL INCLUSION OF SOYBEAN MEAL AS PROTEIN SOURCE IN
TILAPIA FEED AT TWO DIFFERENT PROTEIN LEVELS

Shi-Yen Shiau, Jan-Lung Chuang and Chan-Lan Sun

Dept. of Marine Food Science
National Taiwan College of Marine Science & Technology
Keelung, Taiwan 20224
Republic of China

An experimental feeding trial was conducted with tilapia (Sarotherodon niloticus x S. aureas) to determine whether commercial hexane extracted soybean meal could be used as a partial replacement for fish meal at two different dietary protein levels, 24% and 32%. In each protein level, 30% of fish meal protein was replaced by soybean meal with and without methionine supplementation. Fish meal group was used as control. There were total of 6 dietary groups in the study. The experiment was carried out in a closed circulated filtered rearing system for 2 months. The following results were obtained: Without methionine supplementation, when soybean meal was substituted in diets containing 32% protein, growth and feed conversion ratio of tilapia was reduced significantly ($P < 0.05$) as compared to the fish receiving the control diet. However, the difference between the test and control group was not different ($P > 0.05$) when the diets contained 24% protein. With methionine supplementation, the differences between the test and control groups were not different ($P > 0.05$) despite the protein level in the diet. The protein digestibility, dry matter digestibility and protease activity in the stomach, intestine and liver of tilapia ingesting the fish meal control diet were identical to those noted in fish receiving the test diet regardless of dietary protein content.

These data suggest that without the supplementation of methionine, fish meal can be replaced partially by commercial hexane extracted soybean meal when the dietary protein level is suboptimal for tilapia growth. However, at the optimal dietary protein level (32%), partial replacement of herring meal protein with protein from soybean meal depresses both growth and feed conversion in tilapia.

PROTEIN REDISTRIBUTION DURING SEXUAL MATURATION IN ATLANTIC SALMON

D.F. HOULIHAN*, N. MARTIN*, C. TALBOT⁺ & C. NEWTON*

*

DEPARTMENT OF ZOOLOGY, UNIVERSITY OF ABERDEEN, TILLYDRONE AVENUE,
ABERDEEN AB9 2TN, SCOTLAND, U.K.

+

FRESHWATER FISHERIES LABORATORY, FASKALLY, PITLOCHRY, SCOTLAND, U.K.

Rates of protein loss were measured in various tissues during sexual maturation in atlantic salmon. These were compared with measured rates of protein synthesis in the tissues to construct a balance sheet of protein synthesis and degradation. The relative body composition and rates of protein synthesis of the adult animals have also been compared with the body composition and protein synthesis rates of growing juvenile salmon. During gonad development the gills, liver and red muscle have high rates of protein synthesis and the rate of protein loss from the tissues keeps them in a constant proportion to the total body weight. In contrast the white muscle and stomach have low rates of protein synthesis and show negative allometry. Protein and amino acid transfer from the white muscle to the gonad and other tissues will be discussed.

PROTEASE-DIRECTED ANTIBODIES AND IMMUNOMETRIC ASSAYS AS TOOLS IN
STUDIES OF PROTEIN NUTRITION AND METABOLISM IN FISHES

Knut Hjeltnelund, Ingrid Ugelstad and Jan Arne Arnesen

The Foundation of Applied Research at the University of Tromsø -
FORUT
P.O.Box 3063, Guleng, 9001 TROMSØ, NORWAY

Protein is the major nutrient in the food of most teleost fishes. Hence, we may assume that the nutritional process of extracellular protein digestion is of fundamental importance. In fish larvae and in stomachless fishes the primary digestion is undertaken in the intestine by proteolytic enzymes of pancreatic origin. Among these, trypsin is probably a key enzyme in the regulation and onset of extracellular digestive processes. Mechanistic studies of the production, activation, and release of trypsin into the gut of fish have been hampered, however, by lack of reliable methods to quantify enzyme activity in situ. In particular, with very small subjects as larvae, it is almost inevitable that extracts of the gut will be contaminated with serum or sarcoplasm that contain inhibitors of trypsin. Moreover, the direct enzyme assay does not detect the pancreatic form of the enzyme, and it does not discriminate absolutely between trypsin and other proteases that may be present in a crude extract.

Many of these metodological problems can be circumvented by immunological techniques in which we use spesific antibodies directed against the target enzyme. Antibodies against purified fish trypsin were used in immunohistochemical studies of digestive organs in fish larvae as well as in quantitation of trypsin in cod and herring larvae. Results hereof will be presented and methods discussed in relation to strategies for future research on protein nutrition and metabolism in fishes.

IN VIVO TEST FOR ASSESSING PANCREATIC FUNCTION DURING TREATMENT WITH ENZYME INHIBITORS IN RAINBOW TROUT

Dabrowski, K. and Poczyczynski, P.

Department of Basic Fishery Sciences, University of Agriculture and Technology, 10-957 Olsztyn-Kortowo, Poland

A simple method for the measurement of p-aminobenzoic acid (PABA) in surrounding water following oral administration of N-benzoyl-L-tyrosyl-p-aminobenzoic acid (Bt-PABA), a synthetic peptide used for the assessment of chymotrypsin activity, was investigated.

Fish were fed four experimental diets in which fish meal protein was substituted in 0, 25, 50 and 100% during three months long trial. Significant differences in growth rate of fish were found. Dynamics of the excretion and the total amounts of PABA recovered differed between fish groups.

An In Vitro Method for Measuring Protein Digestibility

A.E.EID & A.J.MATTY

Department of Molecular Sciences, University of Aston,
Birmingham B4 7ET, U.K.

Institute of Aquaculture, University of Stirling,
Stirling FK9 4LA, Scotland, U.K.

A simple and rapid in vitro digestion method was developed which simulates the digestive tract of carp. Protein sources were incubated in vitro with carp intestinal extracts and the apparent digestibility of proteins were determined.

Fishmeal diets containing 14 and 27% protein had apparent digestibilities of 90.2 and 93.0 ; casein (18 and 26% protein) digestibilities of 91.5 and 93.2 ; soybean (10 and 20% protein) 84.2 and 85.3 ; sunflower (8 and 16% protein) 64.2 and 66.1 ; fishmeal + soybean (18% protein) 86.5.

There was a strong correlation between these in vitro digestibilities obtained and in vivo results ($r = +0.99$).

ESTIMATION OF PROTEIN SYNTHESIS IN FISH LARVAE USING
AN IN VITRO RIBOSOME ASSAY

P.E. Hansen, E. Lied and T. Børresen

Technological Laboratory, Danish Ministry of Fisheries
Building 221, Technical University
DK-2800 Lyngby, Denmark.

It has previously been shown that there is a correlation between protein synthesis measured in vitro in muscle from fish and growth.

The purpose of this study was to make the in vitro method more sensitive for use in studies of protein synthesis in fish larvae.

Cod and herring larvae was studied. It will be shown that measurements of amino acid incorporating activity are possible with ribosomes from 50-100 larvae.

As a result of the use of whole larvae and high growth rate, the capacity of incorporating amino acids into protein in cod larvae is about 20 times higher as compared with polyribosomes isolated from cod muscle.

TOTAL VOLATILE NITROGEN - A QUALITY CRITERION FOR FISH
SILAGE ?

Herborg Haaland and Leif R. Njaa

Institute of Nutrition, Directorate of Fisheries
p.o.box 4285, Nygaardstangen
N-5028 Bergen, NORWAY

Fish silage is a suitable ingredient in fish farming when it can be produced from cheap raw material and used in wet pellets by a feed-kitchen situated near the consumer farms. The quality of fish silage depends on the quality of the raw material used, and on proper silaging conditions. The most important of the latter seems to be good control of the pH. In Norway formic acid is almost exclusively the acid used for making fish silage. As total volatile nitrogen (TVN) is used as a quality criterion for consumer fish, for fish meal raw material and for fish meal, it has been suggested also for fish silage.

Fish silage of various raw materials were analysed at different times during storage at room temperature. The NH_3 -part of TVN increased, and an almost parallel decrease was found in amide-N. Amide-N was taken to be the difference between NH_3 -N before and after weak hydrolysis. We contend that TVN or eventually NH_3 -N is not a suitable quality criterion if the pH of the silage is kept below 4.0. In that case NH_3 will mainly be formed from the acid-amide-N of glutamine and probably also asparagine and will thus not affect the amino acid composition of the silage as determined after acid hydrolysis of the sample with 6M HCl.

STUDIES ON THE ESSENTIAL FATTY ACID REQUIREMENT OF CHANNEL CATFISH

R. P. Wilson, S. Satoh and W. E. Poe

Mississippi State University, Department of Biochemistry, Drawer 88,
Mississippi State, MS 39762 (U.S.A.)

Previous workers have been unsuccessful in inducing an essential fatty acid deficiency in channel catfish, thus no essential fatty acid requirement has been established for this species. We have been able to induce an essential fatty acid deficiency by feeding a lipid-free purified diet prepared with ingredients that had previously been extracted with boiling ethanol. In our initial study, catfish were fed the lipid-free diet for a six-week conditioning period. Growth rates of fish fed the lipid-free diet, a 10% tristearin diet and a 10% corn-cod liver oil diet for 15 weeks were 76, 407 and 745% increase in initial body weight, respectively. Fish fed the lipid-free and tristearin diets had higher levels of 20:3n-9 in liver, muscle and whole body lipid. The ratio of 20:3n-9 to 22:6n-3 in the polar lipids of liver, muscle and whole body tissue was less than 0.4 for the corn-cod liver oil diet fed fish but greater than 0.4 for fish fed the other two diets.

Supplementation of 1% 18:3n-3, 1% 18:2n-6 or 1% 18:3n-3 plus 1% 18:2n-6 to tristearin or ethyl oleate based diets (5% total lipid) did not improve the growth rate of catfish to the level obtained by feeding a 5% corn-cod liver oil diet during a 12 week study. These data indicate that the 18:3n-3 and/or 18:2n-6 fatty acids may not be essential for the catfish and that the n-3 highly unsaturated fatty acids contained in cod liver oil may be responsible for the enhanced growth of the fish fed the corn-cod liver oil diet. Therefore, the effect of supplementing diets with n-3 highly unsaturated fatty acids on growth rate is being studied..

ESSENTIAL FATTY ACID REQUIREMENT OF JUVENILE STRIPED JACK,
LONGIROSTRIS DELICATISSIMUS

T. Watanabe, T. Arakawa, T. Takeuchi and C. Kitajima

Professor, Laboratory of Fish Nutrition, Department of
Aquaculture, Tokyo University of Fisheries
Konan 4, Minato-ku, Tokyo 108, Japan

Requirement of juvenile striped jack for w3 highly unsaturated fatty acids (w3 HUFA), the essential fatty acids (EFA) for marine fishes, was determined by feeding them fish meal diets containing different levels of w3 HUFA ranging from 0.7 to 3.5% in diet. Juvenile fish weighing 0.26g in average were fed the experimental diets for 60 days at water temperature of 19.9-24.6°C. After 9 days feeding, a high mortality and poor appetite were observed in fish receiving the diet lowest in w3 HUFA content (0.7%). Fish fed a diet containing 1.2% w3 HUFA also began to show a high mortality at around 55th day of feeding, thus the feeding experiment was terminated at 60th day. No mortality was observed in the fish receiving the 3.5% w3 HUFA diet. Growth of fish and feed efficiency were proportional to w3 HUFA levels in diet and reached plateau at a level of 1.8% in diet.

Feeding the lowest w3 HUFA level diet resulted in increase of 18:1w9, but not of 20:3w9 in whole body polar lipids like other marine species. The 18:1w9 level was reduced by elevation of dietary w3 HUFA levels. The percentage of 22:6w3 was also proportional to dietary w3 HUFA levels, but 20:5w3 was kept nearly constant regardless of the fact that all the diets contained both 20:5w3 and 22:6w3 at almost the same ratio, suggesting bioconversion of 20:5w3 to 22:6w3 or selective utilization of 20:5w3 in striped jack. The requirement of juvenile striped jack for w3 HUFA is estimated to be around 1.8% in diet from these results in both feeding and analyses.

Phospholipid fatty acid retention in cod (*Gadus morhua*) fed reduced dietary n-3 fatty acid levels.

Ø. Lie and G. Lambertsen

Institute of Nutrition, Directorate of Fisheries
P.O. Box 4285, N-5028 Bergen, Norway.

Cod (*Gadus morhua*) fry, mean weight of 0,7 g were fed three diets based of squid mantle mixed with varying levels of total lipid extracted squid mantle and peanut oil compared with a diet based on squid mantle and capelin oil. Thus four levels of n-3 fatty acids were tested.

Increased mortality was observed after 7 weeks in the group fed a diet without n-3 fatty acids. All groups were sampled after a feeding period of 9 weeks. The mortality was then 50% in the group fed no n-3 fatty acids. Addition of cod liver oil to this diet prevented further mortality.

A combined HPLC/ capillary GLC method was used for the determination of the fatty acid composition in the main phospho- lipids (PC, PE, PS and PI) in the cod muscle tissue using 19:0 as an internal standard. Linoleic acid, 18:2 (n-6) was retained mainly in PC and PE in all groups fed peanut oil with a corresponding reduction of 20:5 (n-3) and 22:6 (n-3).

The reduced content of the dietary n-3 fatty acid levels reduced the content of the polar lipids, mainly due to a reduction in the PC content, whereas a relative increase in the PI content was observed. Arachidonic acid, 20:4 (n-6) was retained particularly in the PI fraction.

DIFFERENT FATS IN FEED FOR SALMON, EFFECTS ON FATTY ACID
COMPOSITION IN MUSCLE, LIVER AND HEART.

M.S. Thomassen and C. Røsjø.

Agricultural Research Council of Norway, Institute of
Aquaculture Research, Aas, Norway.

It has been observed that differences in dietary fats may be reflected in the fatty acid composition of rainbow trout and salmon. More detailed information about the processes regulating the metabolism and incorporation of fatty acids derived from feed is, however, needed. In conjunction with an experiment where growth rate and product quality was studied in salmon given different vegetable oil (C. Røsjø and M.S. Thomassen, this meeting), we therefore also included analysis of fatty acid composition in several organs.

The diets used in this experiment contained equal amounts of either soyabean oil, low erucic rapeseed oil or high erucic rapeseed oil, and of capelin oil. The salmon were kept in marine net pens and fed the experimental diets for 4 months (September to January).

In heart phospholipids, all vegetable oil diets gave an increased percentage of w-6 and a reduction in w-3 fatty acids. This was mainly due to changes in the proportion of C20:4 w-6 and C20:5 w-3, while the percentage of C22:6 w-3 was little influenced.

Preliminary results also show effects on fatty acid composition in liver and muscle, which will be discussed in relation to the composition of the dietary fat.

The effect of dietary lipid composition on growth and reproduction of broodstock chinook salmon (O. tshawytscha) in pen culture.

Robert J.J. Roy and David A. Higgs

Department of Fisheries and Oceans, Government of Canada,
West Vancouver Laboratory, West Vancouver, British Columbia.
CANADA. V7V 1N6.

This experiment investigated the role of n-3 and n-6 fatty acid enriched lipid sources, both alone and in mixtures, on broodstock chinook salmon and their progeny. Herring oil and corn oil was used as the source of n-3 and n-6 fatty acids, respectively, in four experimental diets. Each isocaloric casein-gelatin diet contained 16% lipid and had different ratios of herring oil to corn oil. The experimental diets nominally contained 0%, 1%, 2%, and 4% of the diet as n-3 fatty acid. Four duplicate groups of three-year old chinook salmon were kept in sea pens. Each group was fed a different experimental diet from June until late September. Weight and length did not differ significantly among groups at monthly samplings from June to September. At mid-October, 10 salmon from each group were transferred to freshwater tanks and the rest remained in the sea-pens. After 15 days, no mortality was detected in the sea-pens; the mortality of salmon held in freshwater rose linearly from 50% in the 0% n-3 diet to 1 accidental death in the 4% n-3 diet. Salmon in the sea-pens were spawned and the fertilized eggs were incubated in Heath trays. Egg mortality at eye-up and hatching did not differ among diet groups. Egg weight and volume, and swim-up fry weight and length were greatest in the 2% n-3 fatty acid diet group, and least in the 4% n-3 fatty acid diet group. The results on the chinook of our experiment suggest that (1) n-3 fatty acids may be required for broodstock survival in freshwater; (2) lipid makeup of the diet can influence reproductive parameters that are passed on to the progeny; (3) a combination of n-3 and n-6 fatty acids may influence egg quality.

LIPID REQUIREMENTS OF FISHES: THE ENIGMA OF WARMWATER SPECIES

Robert R. Stickney and Ronald W. Hardy

School of Fisheries WH-10, University of Washington, Seattle,
Washington USA 98195 and Northwest and Alaska Fisheries Center,
National Marine Fisheries Service, 2725 Montlake Boulevard E.,
Seattle, Washington USA 98112

Studies over the past two decades have convincingly demonstrated that salmonids and many marine fishes have a dietary requirement for n-3 fatty acids. In at least some instances, for example in rainbow trout, the requirement can be met by providing dietary 18:3n-3. Chain elongation and desaturation to higher molecular weight n-3 fatty acids seems to proceed rapidly. In some of the marine species examined to date, such high molecular weight n-3 fatty acids as 20:5n-3, 22:5n-3, and 22:6n-3 should be provided in the diet as their synthesis from 18:3n-3 proceeds slowly, if at all.

The situation with warmwater fishes is presently more confused. Some species seem to require both n-3 and n-6 fatty acids, though excessive levels of either seem to inhibit proper incorporation of the other and consequently, retard growth. The requirement for n-6 fatty acids in other species has not been clearly demonstrated, but competitive inhibition of n-3 fatty acid utilization by elevated n-6 fatty acid levels appears to operate.

In this paper, we review the literature on the lipid requirements of fish, but concentrate on the research that will be required to determine the dietary requirements of n-3 and n-6 fatty acids in warmwater species.

Effects of Various Feed Energy Levels on Total Fat Content and Fatty Acid Composition in White and Red Muscle from Cultivated Rainbow Trout (*Salmo Gairdneri*).

Kiessling, A., Johansson, L. and Storebakken, T*.

Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, P.O. Box 7046, S-750 07 Uppsala, Sweden

* Institute of Aquaculture Research, N-6600 Sunndalsøra, Norway

From a nutritional point of view it is of great importance that the consumption of fish increases. The unique fatty acid composition of the fat from fish and the antitrombogenic effects of long chained polyunsaturated fatty acids on man makes the Salmonoides with their high muscle fat content especially appreciated.

To study the effects of different feed levels on total fat and fatty acid composition in white and red muscle from Rainbow Trout an experiment was initiated at Aqvaforsk Research Station in Sunndalsøra, Norway. Three groups of fishes were given 100, 50 and 25%, respectively, of the energy level regarded as adequate for the species (Storebakken, 1986 PhD-Thesis). To be able to study the capacity of the muscle to adjust to different energy levels of the feed the fish groups were split and the energy level changed. Fish on the 100% feed level were given 50 and 25%, and fish on the 50% and 25% feed level were given 100% of the adequate feed level. Samples were taken from red and white muscle as well as from depots one and eight months after the change of energy feed levels. Fishes for sampling were selected on weight basis. The experiment is now continued in the sea.

In earlier studies we found that the total fat content and fatty acid composition in white muscle did not change significantly during a starvation period of two months (Johansson, L., Kiessling, A. and Kiessling, K.-H.: NJF, rapportserie, 1987, in press).

In the present study preliminary results show that only fishes changing from the 100% feed level to 25% of the adequate feed level, had a decrease in total fat content of white muscle. The results from the other groups are in agreement with previous results. Thus the total fat content in white muscle does not change despite the difference in feed level and weight of the fishes.

DIFFERENT FATS IN FEED FOR SALMON. INFLUENCE ON GROWTH RATE AND SENSORY PARAMETERS.

C. Røsjø and M. Thomassen.

Agricultural Research Council of Norway.

Institute of Aquaculture Research, Ås-NLH, Norway.

We wanted to investigate the possibility of using vegetable oils in feed for salmon. Rapeseed oil or soyabean oil were exchanged with some of the capeline oil in the feed. Growth rate, mortality, sensory parameters and fatty acid composition in heart and muscle were investigated. The results on growth rate and sensory parameters will be presented.

The experiment was conducted from September 1986 to January 1987. 1800 Salmo salar L. with average weight 246 grams were distributed between 18 marine net-pens. We did not observe any significant differences in growth rate or mortality among the groups. Sensory analysis revealed significant differences on scent, colour shade and colour intensity.

Exchanging 50% of the capeline oil with rapeseed oil or soyabean oil in a salmonid feed, composed of about 22% fat, seems to have no effect on growth rate or mortality in Salmo salar L. The results indicates, however, that some vegetable oils may have an effect on pigmentation of salmon.

REQUIREMENTS OF YAMAME Oncorhynchus masou AND COREGONUS
Coregonus lavartetus M. FOR ESSENTIAL FATTY ACIDS

T. Takeuchi, T. Watanabe, S. Thongrod, S. Satoh, and C. Y. Cho

Laboratory of Fish Nutrition, Tokyo University of Fisheries,
 Konan, Minato, Tokyo 108, Japan.

Feeding trials were conducted to determine the requirements of Yamame Oncorhynchus masou and Coregonus Coregonus lavartetus marena for essential fatty acids (EFA) by feeding them diets containing respectively 18:2w6, 18:3w3, a mixture of 18:2w6 and 18:3w3, and w3 highly unsaturated fatty acids (w3HUFA) at various levels. Fingerlings of Yamame weighing 0.94-0.96g and Coregonus weighing 1.39-1.47g were kept on an EFA-free diet for 3 weeks before the initiation of feeding experiments. They were then randomly divided into 8 lots of 40 fish each in Yamame and 35 fish each in Coregonus and fed the experimental diets for 14 weeks in Yamame and 9 weeks in Coregonus at water temperature of 14-17°C. Low growth and feed efficiency together with high mortalities were observed in both fish species fed the EFA-deficient (5% 18:1w9) and 1% 18:2w6 diets and were effectively improved by feeding w3 fatty acids, such as 18:3w3 and w3HUFA. The highest weight gain and feed efficiency were obtained in Yamame fed 1% 18:3w3 and in Coregonus fed 0.5% w3HUFA, although the supplementation of both 18:2w6 and 18:3w3 (0.5% or 1% each) was also effective for improvement of growth. The hepatosomatic index and lipid content in liver were highest in Yamame fed the EFA-deficient diet. The ratio of 20:3w9/22:6w3 in liver polar lipid fractions was below 0.5 in both species fed diets containing 0.5-1% w3HUFA or 1% 18:3w3. Whereas, the ratio was 0.8-2.7 in those receiving 18:2w6 alone or 0.5% 18:3w3 and as high as 16.7 and 2.7 in Yamame and Coregonus* fed the EFA-deficient diet, respectively. Thus, w3 fatty acids, such as 18:3w3 and w3HUFA, were nutritionally more important than 18:2w6 for both species. However, the EFA efficiency of 18:3w3 and w3HUFA was slightly different between Yamame and Coregonus. The requirements of Yamame and Coregonus for EFA was estimated to be about 1% 18:3w3 and 0.5-1% w3HUFA in diet, respectively.

FATTY ACID COMPOSITION OF *Coregonus muksun*, Pallas. CHANGES DURING INCUBATION, HATCHING, FEEDING AND STARVATION.

Antti Soivio, Marjut Niemistö and Monica Räckström.

Department of Zoology, Division of Physiology, University of Helsinki
Unioninkatu 7, 00100 Helsinki, Finland

Whitefish roe, prior to and after incubation (just before hatching) and larvae (just hatched, starved and fed plankton or dry food) were gaschromatographically analyzed for fatty acids.

During incubation only minor changes in the ratios of fatty acids were observed. The only marked change was the increase in the total ratio of MUFA (mono unsaturated fatty acids) by about 9 %-units while the ratio of saturated fatty acids decreased by 4 %-units. The fatty acid composition was identical in roe just prior to hatching and in newly hatched larvae.

Both in starving and dry diet fed larvae (9 days after hatching) the ratio of MUFAs decreased to the early "roelevel" of about 28 %. In starved larvae the ratio of saturated FAs increased by 7 %-units to 27.5 %. The increase in MUFAs of fed larvae was only 3.5 %-units, but the ratio of PUFAs (poly unsaturated FA) also increased by 4 %-units. The main changes in individual fatty acids took place in decreasing 16:1 and 18:1 and increasing 16:0, 18:0 and 22:6. FAs 15:0, 20:0, 22:1 and 22:2 disappeared totally from the starving larvae.

During the first month of feeding dry diet the ratios of fatty acids 22:1 and 18:2 increased and 20:5 and 22:5 decreased when compared to one week old larvae. The ratios of saturated FAs, MUFAs and PUFAs remained unchanged.

When the larvae were fed (for one month) plankton the ratio of MUFAs decreased to 19 % while the ratio of PUFAs increased by 5 %-units to 46.6 %. The most distinct changes in the FA ratios were seen in 15:0 (+50 %), 18:0 (+100 %), 18:1 (-50 %), 20:1 (-80 %), 18:3 (+400 %), 18:4 (+200 %), 20:4 (+400 %), 22:6 (+70 %) and unidentified (+200 %). In addition the FA 22:1 disappeared and new acids 17:0 and 24:2 appeared during this period.

The FA composition of the whitefish larvae highly resembled that of their food. However, the plankton fed larvae seem to synthesize fatty acids 17:0, 18:0, 20:2 and 20:4, of which the first and the last ones could not be identified in the plankton.

Erythrocyte phospholipid fatty acid composition and haematological values in cod (*Gadus morhua*) held at different water temperatures.

Ø. Lie, E. Lied and G. Lambertsen.

Institute of Nutrition, Directorate of Fisheries
P.O. Box 4285, N-5028 Bergen, Norway.

Cod (*Gadus morhua*) with mean initial weight of 180 g were fed a diet based on squid and capelin oil at 8, 12 and 16 °C for 9 weeks.

The fatty acid composition in the main phospholipids (PC, PE, PI and PS) from the erythrocytes was determined by a combined HPLC/ capillary GLC method.

Reduced levels of saturated fatty acids and increased levels of the polyunsaturated fatty acids (PUFA) in PC, PE and PI were observed with reduced water temperature. Minor differences in the fatty acid composition of PS were found. The increased PUFA in PC and PE was mainly due to an elevated level of 22:6 (n-3), whereas 20:5 (n-3) and 20:4 (n-6) were raised in PI, especially at 8 °C.

Increased number of erythrocytes (RBC) and increased haemoglobin (Hb) contents were found with increasing water temperatures, whereas a reduced middle cell haemoglobin (MCH) and middle cell volume (MCV) were shown at 16 °C compared to 8 °C. The middle cell haemoglobin concentration (MCHC) did not vary at the different temperatures.



INVESTIGATING THE LIPID AND (n-3) HIGHLY UNSATURATED FATTY ACID REQUIREMENT IN SPARUS AURATA LARVAE BY COMPARING THESE FRACTIONS DURING STARVATION AND FEEDING.

W.M. Koven, G.Wm. Kissil and A.Tandler

National Centre for Mariculture
Isreal Oceanographic & Limnological Research
P.O.Box 1212, Elat
88112, Isreal

The gilthead seabream (Sparus aurata) is a species with commercial potential that is presently being developed for cultivation in Israel. The aim of this study was to evaluate the larval lipid class and fatty acid requirement of Sparus aurata. This was done by comparing patterns of accumulation and loss of lipid levels and their fatty acids in feeding (Isochrysis enriched rotifers) and starving (for 6 days) larvae.

Neutral lipid expressed as (micg./mg of wt. larvae) decreased rapidly in both starved (31.0%) and fed (70.7%) fish. In contrast polar lipids (micg./mg of dry wt larvae) of starved and fed fish decreased gradually (16.0% and 13.2% respectively). These lipids play an important structural role in the cellular membrane and are usually preferentially conserved.

The order of utilization in starved fish in the total neutral and polar fractions was (n-6) > (n-9) > (n-3) > (n-3)HUFA. This conservation of the (n-3)HUFA's was strongest in the polar fraction particularly with docosahexaenoic acid, 22:6(n-3). However in feeding larvae polar lipid exhibited a reverse trend in fatty acid utilization where the essential (n-3)HUFA's were the group most drawn upon [(n-3)HUFA > (n-3) > (n-6) > (n-9)].

Docosahexaenoic acid alone decreased 46.8% from day 6 to day 20. This may be interpreted as a direct result of feeding a rotifer low in 22:6(n-3). More interestingly, if there is a biochemical strategy to conserve the essential 22:(6n-3) during starvation then a substantial decrease in its level during feeding suggests that Isochrysis enriched rotifers may not provide sufficient levels of this fatty acid for growing Sparus aurata larvae.

ESSENTIAL FATTY ACID REQUIREMENT OF LARVAL RED SEA BREAM,
PAGRUS MAJOR

T.Watanabe, M.S.Izquierdo and C.Y. Cho

Professor, Laboratory of Fish Nutrition, Department of
Aquaculture, Tokyo University of Fisheries
Konan 4, Minato-ku, Tokyo 108, Japan

Several experiments on the essential fatty acids (EFA) requirements of larval red sea bream were conducted using rotifers enriched by the direct method. Preliminary experiments to verify the amount of w3 highly unsaturated fatty acids (w3 HUFA) incorporated by rotifers permitted the preparation of varieties of rotifers containing different amount of w3 HUFA. 18:1w9, pollock or cuttlefish liver oil and a methyl ester mixture containing 85% of w3 HUFA were used as lipid sources. Three feeding experiments were conducted using 100 L tanks at 18.5-24.2°C. In Expts. I and II larvae were fed the enriched rotifers for 16 and 10 days respectively, from the 3rd day after hatching. In Expt. III, 12 day-old juveniles of 3.88mm T.L. were fed for 14 days. Each experimental group of larvae was fed with one of the varieties of the enriched rotifers containing w3 HUFA from 0.14 to 0.70%.

The larvae fed on rotifers containing a low percentage of w3 HUFA showed a poor growth and a heavy mortality. However, larval growth, survival and results of activity test were improved by elevation of w3 HUFA levels in rotifers and reached a plateau at a level of 0.4%. On the other hand, fish receiving the rotifers fed on the w3 HUFA methyl ester mixture as a sole lipid source showed a poor growth, a low survival rate and a poor response to the activity test, at any levels of w3 HUFA concentration. The levels of w3 HUFA in fish increased gradually as increase of the percentage of w3 HUFA rotifers and reached a maximum at 3%, except in the fish fed the w3 HUFA methyl ester mixture that showed lower percentages of crude lipids and w3 HUFA in fish. These results suggest that a minimum EFA requirement of larval red sea bream for w3 HUFA is about 0.4% w3 HUFA in rotifer.

"THE EFFECT OF ENRICHMENT DIET ON THE FATTY ACID COMPOSITION OF THE ROTIFER *B.Plicatilis*"

RODRIGUEZ RAINUZZO ,JOSE

SINTEF 21 HAVBRUKSENTER

A culture of the rotifer Brachionus plicatilis (200ind/ml 0.5 μ g dry weight/ind) fed it with ω -yeast (capelin oil+ yeast, 10/1 weight) was transferred after 24 h of hunger to 10 different vessels ,each 20 l, and the different diets were added.

The effect of the different diets after 24h feeding is shown on the fatty acid composition.

PARTIAL PURIFICATION AND CHARACTERIZATION OF PANCREATIC
TRIGLYCERIDE LIPASE FROM COD (Gadus Morrhua)

Gjellesvik, D. R., Walther, B. T. & Raab, A. J.

Dept. of Biochemistry, University of Bergen, Norway.
Årstadveien 19, N-5009 Bergen, Norway.
EARN-addr.: MBIDG AT NOBERGEN

Cod pancreatic lipase has been purified and studied with respect to substrate specificity and dependence of cofactors such as colipase and bile salts.

Some authors have suggested that fish pancreatic triglyceride lipases are different from lipases from other sources. These suggestions, however, are made on the basis of studies of crude pancreatic (intestinal) juices, and thus may result from the actions of several enzymes. One aim of this work has been to isolate cod pancreatic lipase for comparison with pancreatic lipase from other sources.

The lipolytic activity in cod pyloric caeca was determined by hydrolysis of purified olive oil in gum arabic emulsion. The purification procedure involved batch chromatography on DEAE-cellulose, ammonium sulphate precipitation, chromatography on DEAE-sepharose fast flow column, and finally gel filtration on Superose 12. By these combined methods pancreatic triglyceride lipase was purified over 200-fold from defatted powder of cod pyloric caeca. Further purification is still in progress.

Preliminary results indicate that lipolysis in this system has an absolute requirement for bile salts, at least at high concentrations of insoluble triglycerides. This is in contrast to the properties of pancreatic lipases from other sources. On the other hand, tributyrin was hydrolysed by partly purified cod lipase in the absence of bile salts, although we cannot exclude that this was caused by other esterases that are capable of hydrolysing soluble substrates.

The above results make it difficult to detect colipase in the preparations studied. However, no preparations of cod lipase were stimulated by porcine colipase in the presence of bile salts. In addition, all preparations of colipase from cod pyloric caeca have been inactive when assayed on porcine colipase-free lipase.

25/8/87 9^h45-10am
DISEASE RESISTANCE AND HUMORAL ANTIBODY PRODUCTION IN
RAINBOW TROUT FED HIGH LEVELS OF VITAMIN C

J. E. HALVER and O. NAVARRE (X)

Get his Thesis *

SCHOOL OF FISHERIES, UNIVERSITY OF WASHINGTON,
SEATTLE, WA 98195 USA

Young rainbow trout(1-10g) fed test diets containing 1,5,10, and 20X maximum growth requirements for L-ascorbic acid and then challenged by injection or dip with Vibrio anguillarum cultures survived at varying rates dependent upon the C intake. Significant differences appeared between dietary treatments in weight, growth rate, mortality and body C content. Resistance to bacterial infection and the humoral antibody production were assessed for the trout on various dietary treatments. Resistance to Bacterial infection was directly related to C intake, and was reflected in improved survival of fish challenged and fed on 5X,10X or 20X normal growth requirement C intake. Humoral antibody production was improved after 3-4 weeks on test, and persisted up to 10-12 weeks. Rainbow trout may survive bacterial challenge and improve humoral antibody production when fed 5X or 10X growth requirement levels of vitamin C.

10-1945 am 20/1/87

THE ROLE OF VITAMIN E IN ATLANTIC SALMON (SALMO SALAR) NUTRITION AND (IMMUNE RESPONSE)

S. P. Lall, G. Olivier, J. A. Hines and H. W. Ferguson*

Biological Sciences Branch, Department of Fisheries and Oceans,
P. O. Box 550, Halifax, Nova Scotia, Canada B3J 2S7
*Ontario Veterinary College, University of Guelph, Guelph,
Ontario, Canada N1G 2W1

A study was conducted to determine the requirement and effects of α -tocopherol on immunity and disease resistance in Atlantic salmon (Salmo salar). Low vitamin E basal diet (5.5 mg/kg) containing herring oil (5%) and tocopherol stripped corn oil (5%), were supplemented with 0, 10, 30, 60, 120 and 240 mg of vitamin E (as DL- α -tocopherol acetate) per kg of diet and fed to triplicate groups of Atlantic salmon fingerlings for 22 weeks. Growth rate was improved by supplementation with vitamin E; increasing vitamin E above the lowest level did not affect the growth rate. There was no difference in food conversion nor mortality rate between all the dietary treatments. Fish fed no vitamin E supplement showed significant low tissue vitamin E levels in muscle, liver and heart and increased erythrocyte fragility. There were no gross symptoms of vitamin E deficiency. Histological examination of groups fed low vitamin E diet showed skeletal muscle and myocardial degeneration.

The following immune responses were also investigated: antibody production, bactericidal activity of serum (complement system) and non-specific resistance to the disease furunculosis caused by Aeromonas salmonicida. For all diets, no effect on the non-specific resistance of Atlantic salmon to furunculosis was observed. Groups of fish fed 0, 30, 60 and 240 mg of vitamin E per kg of diet were also vaccinated with formalin-killed A. salmonicida cells. One month after vaccination, both the humoral response and the complement system were not affected by these dietary levels of vitamin E.

-ve effects
no significant changes

10³⁰

Seals #84 43

RELATIONSHIP BETWEEN BRAIN SEROTONIN LEVEL AND INCIDENCE OF
SCOLIOSIS CAUSED BY TRYPTOPHAN DEFICIENCY IN CHUM SALMON FRY

Toshio Akiyama and Takeshi Murai

National Research Institute of Aquaculture

Nansei, Watarai, Mie 516-01
JAPAN

In order to test the hypothesis that the scoliosis which is known to occur in salmonids fed a tryptophan (Trp) deficient diet may be induced by abnormal and involuntary contraction of the muscle due to depletion of a neurotransmitter, serotonin(5-HT) in the central nervous system, several feeding studies were conducted using chum salmon fry. Diets used were semipurified diets containing various combination of Trp, 5-hydroxy-L-tryptophan(5-HTP, precursor of 5-HT), MK486(inhibitor of 5-HT synthesis in periphery) and DL-p-chlorophenylalanine(PCPA, inhibitor of 5-HT synthesis). Supplementation of 5-HTP(130mg/100g diet) instead of Trp completely prevented incidence of the scoliosis. Combination of 5-HTP (10-50mg) and MK486(1-5mg) increased 5-HT content in the brain and lowered incidence of the scoliosis further than supplementation of 5-HTP alone. On the other hand, supplementation of PCPA(290mg) to Trp sufficient diet decreased the brain 5-HT level and induced the scoliosis. These findings clearly indicate that occurrence of the physical deformity is related to depletion of 5-HT in the central nervous system. In addition, relationship between incidence of the scoliosis and rearing temperature will be discussed.

RATION LEVEL FOR SALMONIDS -
INFLUENCE ON HEALTH AND SEXUAL MATURATION

T. Åsgård*, T. Storebakken*, R. Salte** and G. Andorsdottir**.

Agricultural Research Council of Norway
Institute of Aquaculture Research

* N-6600 Sunndalsøra

** Box 10, N-1432 Ås-NLH

The effect of different ration levels on health parameters and age of sexual maturity were investigated in a series of experiments in Atlantic salmon and rainbow trout. The ration levels varied from min. 25% to max 325% of a standard ration level which was calculated according to expected growth based on fish size and water temperature. The experiments were initiated at start feeding and proceeded until the fish were sexually mature.

Excess feeding did not influence size distribution, gross pathology or mortality directly but may be deleterious to the environment. Adequate feeding resulted in a normal size distribution, few fish with stunted growth and no cannibalism. In salmon parr underfeeding resulted in a greater size variation, aggression, a larger proportion of stunted fish and cannibalism. Mortality is greatly increased if there is severe underfeeding. The aggression was reflected in fin erosion, particularly of the dorsal and pectoral fins. These findings were also observed in fish which had been underfed for only a few weeks. Underfeeding also increases mortality among large salmon in sea cages. The effect of underfeeding on health of rainbow trout was less pronounced than in salmon.

The level of feeding during the first 6 months of the life of salmon did not result in differences in sexual maturation 6 and 18 months after transfer to seawater. However, moderate or strong underfeeding throughout the experimental period affected the age at maturation and reduced early maturation. The severe health consequences of underfeeding are similar to those described for high population densities.

HISTOLOGY OF THE LIVER AFTER CHRONIC ASTAXANTHIN FEEDING
IN SAROTHERODON NILOTICUS AND COLISA LABIOSA

Helmut Segner, Peter Arend, Klaus von Poeppinghausen
Hartmut Schmidt

T e t r a W e r k e
Dr. rer. nat. Ulrich Baensch GmbH
Herrenteich 78
D-4520 Melle 1

?
The influence of chronic feeding (for 23 weeks) of various concentrations of astaxanthin on the liver of Sarotherodon niloticus and Colisa labiosa was investigated histologically. With increasing astaxanthin concentration of the diet (0 - 132 mg/kg food) an improvement of the liver performance was observed. Therefore it has been concluded that xanthophylls, aside from their function as vitamin-A precursors and as pigmenters, have a positive nutritional function in the intermediary metabolism of fish.

INFLUENCE OF DIET AND PEROXIDATIVE RANCIDITY ON FRY OF ATLANTIC AND COHO SALMON

Ketoia, H. George, C. E. Smith, and G. Kindschi

U.S. Fish and Wildlife Service
Tunison Laboratory of Fish Nutrition
3075 Gracie Road
Cortland, NY 13045, USA

Two experiments were conducted to determine the influence of six commercial diets on growth rate and mortality in swim-up fry of Atlantic (*Salmo salar*) and coho salmon (*Oncorhynchus kisutch*). Furthermore, the relationship between peroxidative rancidity of diets and performance of fry was examined. Each diet was fed to triplicate lots of Atlantic salmon (476 fry each) and coho salmon (500 fry each) reared at 9 and 10°C, respectively. In each experiment, all diets were fed at equal levels of dry matter.

Results (Table 1) show that diet significantly influenced weight gains (g/fish) in both species and also significantly influenced mortality in Atlantic salmon but not coho salmon. The reason that these commercial diets caused differential performance may be related to a previously unrecognized high susceptibility of swim-up fry to relatively low levels of peroxidative rancidity in diets. For both species, there were highly significant regression correlations ($r = 0.8$) between weight gains and results of analyses of diets for thiobarbituric acid (TBA) and peroxide value (PV). For Atlantic salmon the relationship between mortality and dietary rancidity was less certain because regression correlation was significant ($P < .025$) for PV ($r = 0.6$) but not for TBA ($r = 0.4$) analyses. This lack of significance may be related to the high variability of the mortality data and analyses of TBA.

TABLE 1: EFFECT OF DIET AND RANCIDITY ON SALMON FRY

Diet (Atlantic salmon)					Diet (Coho salmon)				
8 weeks:					8 weeks:				
#	PV (me/kg)	TBA (mg/kg)	GAIN (g)	MORT (%)	#	PV (me/kg)	TBA (mg/kg)	GAIN (g)	MORT (%)
1	1.9	1.4	.22 ^a	11 ^a	2	8.4	11.0	1.3 ^w	.5
2	8.4	11.0	.14 ^b	24 ^b	3	5.8	12.0	1.6 ^x	.8
3	5.8	12.0	.16 ^b	16 ^a	5	4.9	3.4	1.6 ^x	.5
4	2.3	8.3	.22 ^a	2 ^c	7	1.1	3.3	2.2 ^y	.7
5	4.9	3.4	.24 ^a	11 ^a	8	3.0	1.4	2.1 ^y	.7
6	3.4	8.1	.18 ^c	24 ^b	9	13.3	6.7	1.2 ^z	.9
% Coef. of Variability				6	21	% Coef. of Variability			
						5			

a, b...z Values not followed by a common superscript differ ($P < 0.05$).

IRON AND COPPER REQUIREMENTS OF ATLANTIC SALMON (SALMO SALAR)

S. P. Lall and J. A. Hines

Biological Sciences Branch, Department of Fisheries
and Oceans, P. O. Box 550, Halifax, Nova Scotia, Canada
B3J 2S7

Two experiments were conducted at 15°C to determine the iron and copper requirements of Atlantic salmon (Salmo salar) fingerlings. Purified diet supplemented with graded levels of iron (0, 15, 30, 60, 120 and 240 mg/kg) or copper (0, 2.5, 5, 10, 20 and 40) were fed to triplicate groups of Atlantic salmon for 20 weeks. There was no significant difference in weight gain, feed efficiency and total mortality attributable to the level of dietary iron. Moreover, no disorders and gross deficiency signs were noted. Fish fed basal diet (12.9 mg Fe/kg) with no iron supplement showed a reduction in hematocrit, hemoglobin and serum iron levels and erythrocyte count values as compared with iron supplemented diet. Approximately 30 Mg of iron supplement was required to maintain optimum hemoglobin level and other blood parameters. Mean total body, liver, kidney and spleen iron showed a gradual increase in fish fed diets containing up to 60 mg iron per kg. The results indicate that the iron requirement of Atlantic salmon is approximately 60 mg/kg diet.

In the second experiment, growth, feed efficiency and blood parameters (hemoglobin, hematocrit and erythrocyte count) were not significantly influenced by dietary copper supplementation. The serum copper concentration and liver cytochrome c oxidase activities were significantly lower in the group fed a basal diet (1.2 mg Cu/kg) or the 2.5 mg copper supplement. The copper content of the body and the liver showed an increase with the gradual increase in dietary copper levels. On the basis of these results, the copper requirement of Atlantic salmon was determined to be 5 mg/kg diet. The effects of dietary treatments on iron and copper contents of Atlantic salmon are also discussed in relation to the retention of these elements from the dietary sources in individual tissues.

Aug 26 '89
Am

STABILITY OF ASCORBATE-2-SULFATE IN TROUT FEED
MEASURED BY HPLC

Willy Schüep, Jürg Marmet and Werner Studer

Department for Vitamin and Nutrition Research.

F. Hoffmann-La Roche & Co. Ltd.

CH-4002 Basle

Switzerland

The stability of ascorbate-2-sulfate (AAS) in pelleted fish feed was determined. An HPLC method was used for the measurement of AAS. The compound of interest was extracted with water under the addition of ammonia. After appropriate dilution with an acetate buffer (pH 4) containing 15 % of methanol, an aliquot was injected on a reversed phase column (SHANDON ODS HYPERSIL, 5 μ m; 250 mm length, 4.0 mm I.D.). The mobile phase consisted of an acetate buffer (pH 4) containing 15 % of methanol together with 0.6 g/l of 1,5-dimethylhexylamin as ion-pairing agent. The detector was set at 254 nm. In case that interfering compounds were present in the sample extract, the retention time of AAS was monitored by changing the concentration of the ion-pairing agent. Quantitative determinations were made by comparing the areas of the samples with those given by known concentrations of standards (4.0 μ g/ml). The precision of the method was checked by multiple analysis on a single sample containing AAS in a concentration of 500 mg/kg. The relative standard deviation for ten measurements was calculated to be better than 3.5 %. The method has also been applied successfully for the analysis of AAS in biological material.

The one year stability data reveal an excellent retention rate of the AAS in mash as well as in pelleted trout feed stored at room temperature and at 37 °C.

Schp 6.5.1987

ASCORBATE-2-SULFATE AS VITAMIN C SOURCE FOR
ATLANTIC SALMON (*Salmo salar*)

Kjartan Sandnes, Tom Hansen¹⁾ and Rune Waagbø

Directorate of Fisheries, Institute of Nutrition
P. O. Box 4285, N-5028 Bergen

¹⁾ Aquaculture Station Matre, N-5198 Matredal
Norway

A feeding experiment was carried out to test the effectiveness of ascorbate-2-sulfate (AS) as vitamin C source for Atlantic salmon (*Salmo salar*). Fish which had previously been fed an ascorbic acid (AA) deficient dry practical diet for four months were used in an experimental set up in duplicate groups with the following additions: Group 1: without supplementation of any form of the vitamin; group 2: 500 mg AA/kg; group 3: AS equimolar to group 2; group 4: 5000 mg AA/kg; group 5: AS equimolar to group 4. At the start of this experiment fish weight was 19.9 g and the water temperature was 7.2 ± 3.1 °C during the experiment which lasted 12 weeks.

Growth was recorded and analyses of liver AA and AS were carried out at the start of the experiment, after 6 weeks and after 12 weeks. At the end of the study haematological tests and analyses of vertebral OH-proline were performed. The liver contents (µg/g) of AA (AS) after 12 weeks were:

Group 1: 6 (0); group 2: 166 (75); group 3: 27 (10); group 4: 309 (130); group 5: 85 (42). Partly supported by the haematological findings, these values show that AS on an equimolar basis does not have the same vitamin C effect as AA in Atlantic salmon. This was also confirmed through a recovery experiment from which data will be presented.

AA	AS	Recovery AA	AS
0	0	6	0
500	-	166	75
-	500	27	10
5000	-	309	130
-	5000	85	42

DO CARP LARVAE REQUIRE VITAMIN C ?

Dabrowski, K., Hinterleitner, S., Sturmbauer, C., Wieser, W.

Institute of Zoology, University of Innsbruck, 6020
Innsbruck, Austria

Common carp (*Cyprinus carpio* L.) larvae were fed with experimental flaked diets during an initial period of 11 days at a water temperature of 26°C. Different diets based on yeast and liver resulted in insignificant differences in individual body weight of fish (6.5-8.8 mg) which also were significantly smaller than fish fed live *Artemia* nauplii (33.2 mg). Fish fed dry diets displayed significantly higher mortality (7.8-56.2%) than fish fed live food (0.6%), the former groups showing signs of vitamin deficiency manifested by caudal fin erosion and spinal deformity. Total ascorbate and dehydroascorbate were analysed in both fish and diets. Total ascorbate concentrations in whole fish were significantly lower in the dry diets fed groups (16.5-36.9 $\mu\text{g g}^{-1}$ wet weight) than in the *Artemia* fed group (101.1 $\mu\text{g g}^{-1}$). Dehydroascorbate constituted, on average, 74% and 55.2% of total ascorbate in dry diets and live *Artemia* fed fish, respectively. Low levels of ascorbic acid in the fish body correspond to low levels of this vitamin in the diet. It seems that common carp larvae require vitamin C which is the opposite to what has been reported for juveniles and adults of this species. It is speculated that larval fish do not have the ability to convert the dehydroascorbate present in the diet to the active form of ascorbate.

DETERMINATION OF BIOTIN STATUS IN SALMONIDS BY RADIOASSAY AND PYRUVATE
CARBOXYLASE ACTIVITY IN PLASMA AND LIVER TISSUE

Ronald W. Hardy, Robert T. Cotter, and Edmunds Casillas

Northwest and Alaska Fisheries Center, Utilization Research Division,
2725 Montlake Boulevard East, Seattle, Washington 98112

Large rainbow trout (Salmo gairdneri) fingerlings (106 g) were fed either a complete or a biotin-deficient, semi-purified diet without avidin for 32 weeks. At monthly intervals, fish from each group were sacrificed to obtain liver and plasma, which was analyzed for pyruvate carboxylase activity and for biotin levels by radioassay. The fish were also weighed monthly and examined for clinical signs of biotin deficiency. No differences in growth and feed consumption were observed between groups throughout the experiment despite a six-fold increase in weight. However, during the last month of the of the study, mortality reduced the number of fish in the biotin-deficient group by 50%. No clinical signs of biotin deficiency other than mortality were observed. Liver pyruvate carboxylase activity was not different between dietary groups until 28 weeks of feeding. Plasma biotin levels were reduced in the fish fed the biotin-deficient diet after 4 weeks of feeding compared to the fish at the start of the experiment and compared to fish fed the complete diet. Plasma biotin levels continued to decline in the biotin-depleted fish.

A RE-EXAMINATION OF THE FISHES NEED FOR CHOLINE/BETAINES: A NEW
PHYSIOLOGICAL ROLE IN OSMOREGULATION?

Gary L. Rumsey

Tunison Laboratory and Cornell University
3075 Gracie Road
Cortland, NY 13045-9357, USA

Data will be presented from recently completed studies regarding the choline requirement of trout and the efficacy of betaine as a dietary replacement for choline. Data from studies where the feeding of 2-amino-2-methyl-1-propanol (AMP), an inhibitor of choline/betaine biosynthesis, will be used to compare the efficacy of betaine, choline, and methionine as dietary methyl donors. Additional observations and measurements on the role of betaine as a feed chemoattractant as well as the role of betaine/choline in osmoregulation will be discussed. Preliminary results based on blood osmolytes and salt water stress tests show a potential osmoprotective effect, heretofore not considered when evaluating the nutrient requirements of these animals.

DIETARY CALCIUM, PHYTATE AND ZINC INTERACTIONS IN CHANNEL CATFISH

Delbert M. Gatlin, III* and Harold F. Phillips

Department of Agriculture
University of Arkansas at Pine Bluff
Pine Bluff, AR 71601

*Present Address:
Department of Wildlife and
Fisheries Sciences
Texas A&M University
College Station, TX 77843-2258

Interactions among dietary calcium (Ca), phytate and zinc (Zn) were investigated by feeding eight purified egg white diets containing 0.5 or 2.0% Ca, 0.5 or 1.5% sodium phytate and 20 or 200 mg Zn/kg in a factorial arrangement to triplicate groups of fingerling channel catfish (*Ictalurus punctatus*) in aquaria for 12 weeks. Similar weight gain and feed efficiency values were observed for catfish fed all diets except those which contained both 2.0% Ca and 200 mg Zn/kg. Those two diets appeared to limit feed intake throughout the experiment and thus resulted in reduced catfish performance. Diets which contained either 2.0% Ca or 200 mg Zn/kg in conjunction with the lower level of the other mineral did not impair catfish growth or feed efficiency, thus indicating an interaction between these minerals when both were present at the higher levels. At the end of the feeding trial, zinc concentration of bone from catfish fed the various diets was determined to provide an index of zinc bioavailability. Catfish fed diets containing calcium, phytate and zinc levels (abbreviated % Ca\% phytate\mg Zn/kg) of .5\1.5\20, .5\1.5\200, 2\1.5\20, and 2\1.5\200 had mean bone zinc concentrations of 183.3, 126.9, 191.4, and 74.8 ug Zn/g fat-free, dry bone, respectively. In this series of diets, the higher phytate level alone caused a significant ($P < 0.05$) reduction in catfish bone zinc; whereas, the higher calcium level alone did not result in reduced bone zinc. However, a combination of high phytate and high calcium significantly reduced catfish bone zinc to the lowest level. Catfish fed the second series of diets with calcium, phytate and zinc levels of .5\1.5\200, .5\1.5\200, 2\1.5\200 and 2\1.5\200 had mean bone zinc concentrations of 280.7, 307.3, 292.5 and 310.4 ug Zn/g fat-free, dry bone, respectively. These bone zinc levels were significantly higher than those produced by the first series of diets. At the higher dietary zinc level, changes in calcium and phytate did not affect bone zinc levels since excess dietary zinc was available. Results from this study indicate that phytate reduces zinc bioavailability to the channel catfish and this effect is exacerbated in the presence of high dietary calcium.

PIGMENTATION OF SALMONIDS. INTERACTIONS OF ASTAXANTHIN AND
CANTHAXANTHIN ON PIGMENTDEPOSITION IN RAINBOW TROUT

Ole J. Torrissen

Institute of Marine Research
Matre Aquaculture Station
N-5198 Matredal, Norway

Two carotenoid pigments are commonly used to pigment the flesh of farmed salmonids. Astaxanthin, the pigment found in wild salmonids, and canthaxanthin.

In this experiment 6 groups of 74 individually labeled rainbow trout were fed 6 diets formulated to contain 200 mg/kg of astaxanthin and canthaxanthin and where the astaxanthinratio ranged from 0 to 1 by 0.2.

The experiment shows that astaxanthin are absorbed and deposited at a higher ratio than canthaxanthin and that a combination of both pigments (65% astaxanthin and 35% canthaxanthin) give the highest total carotenoid level in the flesh.

This together with the astaxanthin-canthaxanthin ratio in different pigments of the intestinal tract and in plasma indicates that the absorption and deposition mechanism of astaxanthin and canthaxanthin to some extent are independent

The deposition of astaxanthin and canthaxanthin shows large individual variation, from 0.6 to 5.6 mg/kg flesh at the end of the experiment. It is shown that this variation is mainly due to difference in fish size and growth rate. Rainbow trout below 80-90 g deposite carotenoids poorly in the flesh. At low and medium growth there is a linear relation between growth and level of carotenoid in the flesh, but at higher growth this levels off.

NUTRITIONAL AND PHYSIOLOGICAL EFFECTS OF DIETARY NaCl ON RAINBOW TROUT

N.A.Salman and F.B.Eddy

Department of Biological Sciences , University of Dundee
Dundee, Scotland.

In freshwater rainbow trout, high levels of dietary NaCl (9.2-11.6%) caused growth impairment only when the added salt diluted other nutrients of the commercial diet. This was associated with a reduction in food conversion efficiency caused by poor protein efficiency ratio. Food intake was not affected by the added salt and therefore additional amount of diet should be offered to the salt-fed fish to compensate salt dilution. Fish fed isonitrogenous / isocaloric diet of different salt content, however, showed similar growth rate and conversion efficiency. In both diets, low level of salt (4.5%) proved to have a beneficial effect on growth.

A few (3-4) weeks of feeding fish on diet containing 9.2 and 11.6% NaCl appeared to increase the survival of young (10-40g) and larger sized rainbow trout upon transfer to seawater.

Dietary NaCl in excess of 4% caused lower digestibility and faster evacuation rate. Efficient absorption of dietary salt was noticed in freshwater and seawater-adapted rainbow trout. Plasma Na^+ and Cl^- increased progressively during the first 24 hours after feeding with salt-fed fish having the highest level. Retention of normal plasma ionic content 48 hours after feeding indicates the presence of an efficient salt excretion mechanism.

Among the known salt output routes, branchial ionic efflux was found to be the major route for the extrusion of dietary salt load, contributing to about 81% of the total Na^+ efflux in fish fed 11.6% salt in diet. Differences between normally and salt-fed fish in the amount of ions excreted through the faeces were not significant, indicating a minor role for faecal ionic excretion route. A noticeable increase in numbers of chloride cell (about 10%) and gill Na^+/K^+ -ATPase activity (about 30%) was observed in salt fed fish, indicating the possible stimulation of an active salt elimination mechanism during freshwater residence.

DEFICIENCY OF VITAMINS C AND B₂ CAUSED HIGH MORTALITY OF RAINBOW
TROUT FRY

Teskeredžić, Zlatica; Teskeredžić, Emin; Križanac, Vanči

Dr. Sc. Teskeredžić Zlatica
"Ruder Bošković" Institute
Center for Marine Research
Bijenička c. 54
41000 ZAGREB
YUGOSLAVIA

In the course of 1982, 40-80% of the fish of 5-20 g weight size died on many Rainbow trout fry farms.

15-20 weeks after the beginning of feeding period, the fish were found swimming without coordination, gathering at the water inlet and outlet, the heads turned towards the water outlet. The skin was distinctly dark and the spine deformed. Some fish had exophthalmos. On examining the fish, the majority of them had cataract in one or both eyes with atrophy of the lense. Internal organs were pale, sprinkled with tiny bleedings. Apart from the bleeding, in the liver were found smaller whitish areas.

Virusological, bacteriological and parasitological analyses of the fry determined no infectious disease.

Water quality corresponded to the water required for the cultivation of salmonid fry.

Detailed food analysis showed that the feed was lacking in vitamin C and that vitamin B₂ content of the feed was considerably lower than the content declared by the manufacturer.

When vitamin premix was added to the feed (2%), mortality of the fish ceased completely within 30 days.

"Black" and deformed fish were found on the rest of the farms which continued to use this feed during all the rearing.

ASKORBYL PALMITATE AS A DIETARY VITAMIN C SOURCE FOR RAINBOW
TROUT (*SALMO GAIIRDNERI*)

Sissel Albrektsen, Øyvind Lie and Kjartan Sandnes

Institute of Nutrition
P.O. Box 4285
N-5028 Bergen, Norway.

The vitamin C activity of ascorbyl palmitate was investigated for the use in salmonid diets. Three groups of "swim up" rainbow trout (*Salmo gairdneri*) fry were fed identical basal diets for 12 weeks supplemented with no form of ascorbic acid (group A), 600 mg ascorbic acid/kg (group B) and ascorbyl palmitate in equimolar amounts to group B (group C). The fish in group C showed reduced growth the first 8 weeks. From week 8 onwards growth rate in group C paralleled that in group B, while the fish fed the diet devoid of any form of vitamin C (group A) showed reduced growth rate. The concentrations of ascorbic acid at the end of the experiment were for groups A, B and C respectively: < 0.5, 64 and 55 µg/g in whole fish and < 0.5, 156 and 118 µg/g in the liver. Vertebral collagen in fish fed ascorbyl palmitate contained similar levels of hydroxyproline as did fish which were given ascorbic acid as vitamin source. An *in vitro* assay demonstrated the presence of enzymatic activity in the rainbow trout gut hydrolyzing the ascorbyl palmitate.

DETERMINATION OF ASCORBIC ACID AND ASCORBATE-2-SULFATE
IN FISH TISSUES

Kjartan Sandnes, Ruth Waagbø and Rune Waagbø

Directorate of Fisheries, Institute of Nutrition
P. O. Box 4285, N-5028 Bergen
Norway

A classical colorimetric method for the assay of ascorbic acid (AA) is the coupling of its oxidized form, dehydroascorbic acid (DHA) with 2,4 dinitrophenylhydrazine (DNPH). This procedure, commonly termed the Roe and Kuether method, gives the bis-2,4-dinitrophenol hydrazone of DHA which can be detected spectrophotometrically at 520 nm. By increased hydrolysis time and temperature this principle has also been used to determine ascorbate-2-sulphate (AS) (Tucker and Halver, 1981). A direct colour reading of the complex at 520 nm can be influenced by interfering substances in the sample extracts resulting in reduced specificity. An approach to solve this problem have been made (Schüep, Vuilleumier, Gysel and Hess, 1984) with the application of HPLC.

The present paper reports on the extension of the DNPH method with HPLC separation described above also to include analysis of AS. The method has been applied in studies on AS metabolism in fish including analyses of fish tissues and fish feeds.

SOME ASPECTS OF ASCORBIC ACID DURING VITELLOGENESIS
IN RAINBOW TROUT (*Salmo gairdneri*).

Rune Waagbø and Kjartan Sandnes

Directorate of Fisheries, Institute of Nutrition
P.O. Box 4285, N-5028 Bergen
Norway

Two groups of adult rainbow trout were fed diets devoid of ascorbic acid (AA) (Group 1) and supplemented with 2000 mg AA/kg (Group 2) for 18 months including the maturation cycle examined. Haematological data and blood serum levels of oestradiol and vitellogenin were recorded during maturation. Further the content of AA and ascorbate-2-sulfate (AS) were determined in ovaries, liver, spleen, heart and cerebrum.

Neither increased mortality nor macroscopic signs of avitaminosis C was observed in group 1 in the course of the experiment. The fish in this group showed low levels of AA and considerable levels of AS, indicating a conservation of AA as AS in the tissues of adult fish.

Blood haemoglobin and haematocrit were significantly reduced in group 1 fish at the end of the experiment. Also the levels of oestradiol and vitellogenin in the blood were significantly reduced in the fish without dietary supplementation of AA. The results indicate that AS is not mobilized fast enough from the stores to meet the demands of AA for metabolic purposes during maturation in rainbow trout.

A COMPARISON OF GROWTH RATE AND TRACE ELEMENT ACCUMULATION
IN SALMON FRY SUPPLIED FOUR DIFFERENT COMMERCIAL DIETS
UNDER REALISTIC CONDITIONS IN A LARGE HATCHERY

Amund Maage¹, Harald Sveier² & Kaare Julshamn¹

- 1) Institute of Nutrition, Directorate of Fisheries,
Lars Hillesgt. 26, N-5028 Bergen, Norway.
- 2) A/S Saevareid Fiskeanlegg, N-5674 Saevareid, Norway

The very soft water in Western Norway, where most of the commercial hatcheries for the salmon fish farms are situated, make the diet an important source of minerals and trace elements for growing salmon.

This study reports on the effect of dietary element concentrations on the contents of the elements in growing salmon fry. Four different commercial start-diets for salmon were chosen and analysed for major food components, calcium, vit C and the trace elements Zn, Fe, Cu, Se and Cd. Only minor differences were found for the major nutrients in the diets, whereas vitamin C, calcium and trace elements varied up to four times.

After hatching salmon fry were distributed in 24 different tanks approximately 78.000 fry in each. Six tanks were given the same diet. Random samples of 50 fish were weighed weekly. After 5 weeks of feeding, four fish from each tank were weighed, captured and acid digested for trace element analysis.

The levels of calcium and trace elements in the fry were significantly correlated to the elements in the diet. In the total material only zinc showed correlation between concentration in the fry and the body weight.

Most likely all diets tested contained sufficient of the analysed trace elements for optimal growth. Whether these levels are sufficient for optimal health need further investigations.

MAGNESIUM REQUIREMENT OF GUPPY (*POECILIA RETICULATA*
PETERS)

K.E. SHIM AND S.H. NG

Department of Zoology, National University of
Singapore, Lower Kent Ridge Road, Singapore 0511,
Republic of Singapore.

The requirement of guppy (*Poecilia reticulata* Peters) for dietary magnesium was investigated by feeding them with diets containing varying levels of magnesium (0.0, 0.18, 0.36, 0.54, 0.72, 0.90 g/kg magnesium of dry diet) for about three and a half months. Poor growth, low feed efficiency, high mortality were demonstrated in magnesium-deficient guppies. Histopathological signs were also seen in the various organs (kidney, heart, liver, gill, muscle) of these affected fish. Tissue calcinosis, a striking symptom in magnesium deficiency, was only observed in fish fed with 0.0 and 0.18 g/kg magnesium. Mineral contents such as that of magnesium, sodium, calcium and potassium were analysed in the above-mentioned organs. A major trend of low magnesium, potassium and high calcium, sodium concentration was seen in most of the organs analysed in the magnesium-deficient fish. The minimal magnesium requirement elucidated was 0.36 g/kg magnesium of the dry diet if no tissue calcinosis is to occur. The optimal level of magnesium required for optimal growth was found to be 0.54 g/kg magnesium of the diet.

UTILIZATION OF GRAIN NUTRIENTS BY SALMONIDS

Ashild Krogdahl, Einar Watne and Kjell Harald Lunde

Institute of Aquaculture Research, N-1432 As-NLH, Norway

Barley, oats, wheat, and corn, raw, boiled, and extruded were fed to rainbow trout. The grains comprised 40 % of the dry feed ingredients. Fish meal and capelin oil were included at 49 and 10 %, respectively. Chromic oxide, serving as indigestible marker, was added at 1 % level. The diets were prepared as moist pellets with about 40 % water.

Digestibility experiments were carried out with fish kept in two-cubic meter tanks. Each tank contained 19 fish at average weights of 300 g. The fish was fed *ad libitum* for 17 days in each trial. One tank was used per treatment. Contents of the large intestines were collected and pooled from anaesthetized fish in each of the tanks, in three replicates.

The carbohydrate fractions, comprising the major parts of the grains, showed significantly different digestibilities. Wheat showed, on average, the highest carbohydrate digestibility, followed by oats, barley, and corn. The heat treatments affected the carbohydrate digestibilities significantly. The carbohydrates of raw grains were poorly digested. Boiling improved the digestibility of total carbohydrates from an average of 2 % to about 37 %, whereas for extruded grains, the digestibility was 55 %. Fish fed diets containing oats, digested dietary fat less efficiently than fish fed other diets. The heat treatments did not affect the fat digestibilities of the diets. No significant differences were found between grains or grain treatments regarding nitrogen utilization.

GROWTH RATES AND CONVERSION EFFICIENCY DURING
COMPENSATORY GROWTH OF ARCTIC CHARR

Miglaivs, Ilona & Jobling, Malcolm

University of Tromsø, Institute of Fisheries
Dramssveien 201 p.o. box 3080 Guleng
N-9001 Tromsø, Norway

The phenomenon of compensatory growth was investigated in Arctic charr (*Salvelinus alpinus* L.). In order to avoid the effects of social interactions on feed intake and growth, fish were held in isolation. During a period of 8 weeks, the restricted fish were supplied with 10 % of the food consumed by control fish and this ensured that restricted fish either maintained body weight or showed very low rates of growth. The restricted fish were then switched to satiation feeding for a further period of 8 weeks. Food intake of each individual was monitored. Fish were weighed every other week for the determination of growth rates. "Restricted-satiation" fish were sampled at 8, 12 and 16 weeks and control fish at 16 weeks. Proximate body composition was determined and RNA:DNA ratios of muscle and liver analysed. "Restricted-satiation" fish displayed high rates of growth during the realimentation period and food conversion efficiency was high. Attempts were made to relate RNA:DNA ratios to individual rates of growth.

A BEHAVIOURAL METHOD TO TEST FEEDING RESPONSES OF FISH TO
PELLETED DIETS.

Drs. L. Stradmeyer

Frappak Feeds
c/o Freshwater Fisheries Laboratory
Faskally, Piltichry
PH 16 5LB Perthshire
Scotland

Hatchery -reared Atlantic salmon fry, (*Salmo salar* L.), in a test flume, were offered a standard dry pelleted food and a new moist pelleted food, both of the same particle size, but differing in shape, texture and gustatory characteristics. Feeding responses recorded were:

1. Orientation (head and eye movement of fish).
2. Approach (swimming of fish towards pellet).
3. Capture (fish take pellet in mouth).
4. Rejection (fish spit pellet out)
5. Ingestion (fish swallow pellet).

Results showed differences in reactions to and consumption of the two foods. Shape was identified as a major factor in the capture of the food while taste and texture influenced ingestion. The new moist Frappak pelleted diet was preferred and taken twice as often as the standard dry diet.

STUDIES OF APPETITE AND FOOD INTAKE IN ARCTIC CHARR

Even H. Jørgensen and Malcolm Jobling

IFF, University of Tromsø, Box 3083 Guleng, 9001 Tromsø

Quantitative evaluation of gut content in living fish has been determined using a radiographic method. Fish which had been offered a diet labelled with small (125 - 180 μm) iron particles (0.5 % w/w) were X-rayed and gut content quantified by counting the number of particles. The method eliminates problems associated with force feeding, autopsy or pre- and postfeeding starvation. To evaluate the applicability of the method we tested whether the "calculated" gut content (from X-ray analysis) reflected the "real" content at different intervals after a meal. After 7 days of starvation, 190 Arctic Charr (25 g) were given labelled food for 3 hours, after which no food was offered until the end of the experiment. At regular intervals after the meal, a number of fish were killed and weighed, stomach and intestine removed and X-rayed before the contents were removed for drying and weighing. The "calculated" and "real" gut contents could be compared and evaluation time estimated. Until 9 hours postfeeding, the "calculated" and "real" gut contents did not differ significantly. Later on, the X-ray method overestimated stomach content, probably due to preferential retention of the X-ray dense particles in the stomach. The method seems well suited for food intake studies, but unsuitable for evacuation studies, at least in small Arctic Charr.

The method has been used in a pilot study of diurnal rhythmicity of food intake. Under natural light conditions (spring) food intake was measured for 3 hours intervals throughout the whole 24 hours cycle. Food intake varied markedly between intervals, being lowest at mid day/-night (0.5 mg.g fish⁻¹) and highest during the period following sun set (5.15mg.g fish⁻¹). The method appears to be suitable for studies of this kind.

EFFECTS OF CARBOXYMETHYLCELLULOSE ON GASTRIC EMPTYING TIME,
DIGESTION AND GROWTH OF TILAPIA (Sarotherodon niloticus x S.
aureas)

Shi-Yen Shiau, Hao-Ling Yu, Sophy Hwa, Shih-Yang Chen
and Song-Ing Hsu

Dept. of Marine Food Science
National Taiwan College of Marine Science & Technology
Keelung, Taiwan 20224
Republic of China

Carboxymethylcellulose (CMC) was incorporated into experimental rations at four levels (2%, 6%, 10% and 14%) and fed to male tilapia (Sarotherodon niloticus x S. aureas) for 9 weeks. The experiment was conducted in a close circulated filtered rearing system. The following results were obtained: Tilapia fed diets containing 6%, 10%, and 14% CMC showed significantly lower ($P < 0.05$) feed conversion rate than that of fish fed 2% CMC. The dry matter digestion coefficients of the diets generally declined as CMC level increased. However, the differences were not significant ($P > 0.05$). Gastric emptying time increased as the inclusion of CMC in the diet increased. Stomach-body weight ratio, crude protein content and ash content of fish were similar in all the dietary groups. Fish fed 6%, 10% and 14% CMC containing diets had significantly lower ($P < 0.05$) lipid content than that of fish fed 2% CMC diet.



CARBOHYDRATE AS AN ENERGY-SOURCE TO COD - GADUS MORHUA

Hemre, G-I., Lie, Ø., Lied, E., Lambertsen, G.

Directorate of Fisheries, institute of Nutrition
P.O.Box 4285, 5013 Nygårdstangen - Bergen
Norway

Four parallel groups of 50 cod (200g) were given increasing amounts of dextrinized potato starch in their feed. 3.5% mortality were registered during the 8 weeks of the experiment. Protein energy between 50% and 80% was substituted for carbohydrate energy, while fat energy was kept constant at 20%, as earlier experiments had shown that 20 energy% of fish oil sufficed to keep liver size/body size of cod constant. Digestibility was evaluated using Cr_2O_3 as a marker. Plasma glucose was determined using a hexokinase-UV-method. Glycogen content of muscle and liver were measured using an enzymatic method with amylase and amyloglucosidase.

Cod, which is an carnivorous species, had a digestion capacity for dextrinized potato-starch ranging from 30% to 45% of the amount in the feed, increasing amounts of starch giving decreasing digestibility. This is in agreement with experiments with yellowtail, *Seriola quinqueradiata* (Shimeno et al., 1977). The digestibilities of protein and fat seemed independent of the starch content in the feed. The energy intake increased with the starch content in the diet. PER and PPV were the same for all groups. Plasma glucose increased from 35mg/dl to 75mg/dl as the starch energy content in the feed increased from 0 to 30%, showing significant correlation, inspite of wide variations between individual values. The glycogen content in liver and muscle tissue were dependent on the starch content in the feed up to 20 energy% of starch given. At higher starch contents in the feed, maximum levels of 0.5% of muscle wet weight and 3.5% of liver wet weight were found.

FEED AS THE CAUSE OF HIGH MORTALITY OF RAINBOW TROUT IN YUGOSLAVIA

Teskeredžić, Emin; Teskeredžić, Zlatica; Malnar, Leon

Dr.Sc. Teskeredžić Emin
"Ruder Bošković" Institute
Center for Marine Research
Bijenička c. 54

41000 ZAGREB - YUGOSLAVIA

In 1984, on many Rainbow trout farms, 70-80% of the fry died. Significant increase in mortality was observed in the second or in the third week of the feeding period. Some of the fish were found close to the pond wall, the heads turned towards the water inlet, the swimming occasional, irregular and uncoordinated, opercula dilated and the respiration aggravated. Some fry were found with enlarged abdomen and exophthalmos; some were cachexic, lying alive at the bottom of the pond. The liver was yellow and of loose consistency, the intestines filled with yellow substance. On cutting the enlarged abdomen of the fish, clear liquid was leaking out from the peritoneal cavity.

Virusological, bacteriological and parasitological analyses of the fry determined no infectious disease.

Physico-chemical analyses showed that the water quality couldn't have caused the mortality.

It was found by feed analysis that the mortality was due to the inferior composition of the feed.

Microscopic analysis showed that the feed contained 10% wheat, 15-20% fish meal, 10% blood meal and 60-65% meat and bone meal. With different sort of feed, a small number of fish recovered (15-20%), and not without lasting consequences in later growth.

FEEDS AND FEEDING IN FISH CULTURE IN THAILAND

Dr. Kok Leong WEE

Associate Professor
Div. of Agricultural & Food Eng.
Asian Institute of Technology
P.O. Box 2754
Bangkok 10501
Thailand

Aquaculture in Thailand is very diverse with more than 20 species of fish and shellfish being farmed in systems which are categorised as highly intensive with precise controls in management and feed inputs through to those classified as extensive with relatively few or no inputs. Feeds used ranged from complete dry pellet to supplementary feeds in fertilized ponds to the more 'ambiguous' feed/fertilizer inputs. This paper summarizes the wide range of feeds and feeding practices currently in use in fish culture in Thailand.

RATION LEVEL FOR SALMONIDS -
INFLUENCE ON GROWTH, FEED CONVERSION AND BODY COMPOSITION

T. Åsgård*, T. Storebakken*, E. Asutreng** and P.H. Vabø**.

Agricultural Research Council of Norway
Institute of Aquaculture Research

* N-6600 Sunndalsøra

** Box 10, N-1432 Ås-NLH

Different ration levels for Atlantic salmon and rainbow trout, in freshwater and seawater, are being investigated in a series of experiments where we examine growth, feed conversion and body composition. A standard ration level is calculated according to expected growth. One kg of dry feed is offered per kg of expected growth. Other groups of fish receive rations between 25% and 325% of the standard ration.

The salmon reached a mean weight of 28 g after 7 months (2 400 day degrees (d.d.)) and over 180 g after 15 months (4 000 d.d.); survival was above 80% from the eyed egg stage on. About 15 MJ of metabolizable energy or 1 kg of dry feed per kg growth is sufficient for achieving this rapid growth. More feed does not alter the growth or the body composition of the fish but does increase the pollution. Underfeeding results in retarded growth, increased variation in fish size, and on average a leaner fish. The effect of ration level on growth are very similar in freshwater and in saltwater.

The experiments on rainbow trout also demonstrate that overfeeding is unnecessary for rapid growth. Nitrogen digestibility is not significantly influenced by ration level. Protein retention is over 45% in the rapidly growing fish. The experiments demonstrates the necessity of an exact feed ration according to expected growth.

EFFECT OF DIFFERENT DIETS ON EGG PRODUCTION AND QUALITY OF ATLANTIC SALMON

Päivi Eskelinen

Finnish Game and Fisheries Research Institute
Laukaa Fish Culture Research Station
SF-41360 Valkola
Finland

Four-year old Atlantic salmon groups were fed with different feeds. These included 5 dry and 5 semimoist feeds and smelt as a fresh feed. The feeds differed according to their moisture, amount and composition of fat and contents of alpha-tocopherol, retinol, canthaxanthin, ascorbic acid and selenium. The fishes were stripped and fertilized individually and eggs of six females of each feeding group were incubated separately. Growth, gonadosomatic index, fecundity and egg volume and egg size of every female were measured. Survival rates to eying, hatch and first feeding and size fry were determined for each feeding group.

Greatest growth of broodstock fishes was obtained when using fat-supplemented semimoist feed. Volume of eggs/kg and GSI were on an average greater for semimoist feeds (0.284 l/kg and 22.3 %) than for dry feeds (0.250 l/kg and 20.5 %) and for fresh feed like semimoist feeds (0.272 l/kg and 22.9 %). The diameter of swelled eggs changed between the groups only a little.

Relative fecundity was highest for semimoist feeds (mean value 1839 eggs/kg), lowest for dry feeds (1697 eggs/kg) and for fresh feed between these two (1732 eggs/kg).

Survival before eyed stage ranged from 75.9 % to 97.5 %. In this experiment high alpha-tocopherol content in feed didn't increase survival of eggs to eyed stage but high vitamin-C contents may have such an effect.

In most of the measured parameters the groups internal variation was conspicuous, partly because the fishes were first time spawners. The percentage which hatch and reach first feeding stage are discussed, as well as the quality of fry.

GUT BACTERIA IN FISH

Anne Mette Onarheim and Jan Raa



Institute of fisheries, PO Box 3083 Guleng, N-9000 Tromsø

Do fish, like warmblooded animals contain a specific intestinal microflora? How will this microflora eventually be affected by feed composition and by antibiotics? Do intestinal bacteria in fish play a role in the physiological function of the gut and in the resistance against pathogens? These are the major questions dealt with in the project.

Experiments have demonstrated that fish indeed contain a unique microflora intimately associated with the gut mucosa and in the *pylorus cecae*. Wild cod, herring and saithe from an unpolluted marine environment as well as farmed salmon and char contain a characteristic microflora. This flora consists of slow growing, G-negative and pleomorphic bacteria which are catalase positive, facultatively anaerobic and vigorous acid producers also under highly aerobic conditions. Isolates of this "gut bacterium" from the different fish species differ slightly in biochemical properties but they all resemble marine vibrios. They differ however from the species which are known to be pathogenic to fish.

One of the isolates of these "fish gut bacteria" have the ability to produce substances which act antagonistic to *Vibrio salmonicida*, which causes the "Hitra disease", and to other fish pathogenic vibrios.

EFFECT OF DIETARY CARBOHYDRATE ON THE LEVEL OF KETONE BODIES IN THE BLOOD OF SALMONIDS

G. Andorsdottir* and T. Åsgård**

Agricultural Research Council of Norway
Institute of Aquaculture Research

* Box 10, N-1432 Ås-NLH

** N-6600 Sunndalsøra

The objective of this experiment was to examine whether salmonids produce ketone bodies in response to a carbohydrate-free diet. Ketone bodies are alternative energy substrates to glucose in muscle and brain. The precursors of ketone bodies, free fatty acids, are toxic in high concentrations, have very limited solubility and readily saturate the carrying capacity of the plasma albumin. In the contrast, ketone bodies are very soluble, have low toxicity, are tolerated at high concentrations, diffuse rapidly through membranes and are quickly metabolized to CO_2 and H_2O .

Large Atlantic salmon were fed moist diets with or without carbohydrate added to the binder meal premix. After 5 months of feeding, blood-samples were taken and the plasma analysed for acetoacetate using an autoanalyser.

→ The results showed differences in acetoacetate level due to carbohydrate content in the feed. The mean acetoacetate concentration in the blood plasma of fish receiving moist feed without carbohydrate was 23.4 $\mu\text{U/l}$, while the acetoacetate concentration of the fish receiving moist feed containing carbohydrate (approximately 15% of the metabolizable energy) was 13.1 $\mu\text{U/l}$.

CBH → ↓ ketone bodies

EFFECTS OF PIGMENTS FROM DIFFERENT SOURCES ON COLOUR CHANGES
AND GROWTH OF RED TILAPIA NILOTICA

DR. MALI BOONYARATPALIN
MRS. NANTHIYA UNPRASERT

NATIONAL INLAND FISHERIES INSTITUTE
KASETSART UNIVERSITY CAMPUS
BANGKHEN, BANGKOK 10900
THAILAND

The investigation was aimed to determine the effect of pigment sources on colour development, growth, feed conversion and survival of Red Tilapia. The experiments were conducted in 50 x 100 x 50 cm. aquaria, using four pigment sources; spirulina, toreador marigold petal meal, shrimp waste and turmeric. These pigments were added to the basal diet at 10%, 5%, 15% and 5% respectively. The four test diets and the basal diet (control) were fed to Red Tilapia for eight weeks. It was found that fish fed with spirulina, marigold petal meal, shrimp waste, turmeric and basal diet produced single red, flame, flame. orange and orange coloration in Red Tilapia respectively. Snout and base of the fins of fish fed marigold petal meal added diet developed temple yellow coloration. Growth was stimulated by shrimp waste and inhibited by turmeric. Feed conversion was not significantly different from the basal diet except the turmeric added diet. Survival rate was essentially equal for all test diets and not significantly different from the basal diet.

NATURAL ASTAXANTHIN AS A SOURCE OF PIGMENT IN FEED
FOR SALMONIDS.

Bodil Riising.

Grindsted Products A/S
Edwin Rahrs Vej 38
DK-8220 Brabrand
Denmark

An industrial production of Astaxanthin for fermenting the yeast has now been developed.

It has for years been a wellknown fact, that salmonoids are unable to synthesize carotenoids themselves and that the pink colour characterizing wild salmon is due to ingestions of several types of natural feed - phytoplankton and crustaceae - containing different types of carotenoids of which Astaxanthin (3,3-Dihydroxy- β , β -carotene-4,4'-dione) is the most important.

In order to obtain the desired red colour in pen-reared salmonoids, different sources of pigment have been used - shrimp waste, shrimp meal, crill meal, tobis oil.

The greatest importance has been gained by the synthetically produced Cantaxanthin (Carophylred containing 10% Cantaxanthin) produced by Hoffmann La-Roche. Recently Hoffmann La-Roche have also brought a synthetic Astaxanthin product (Carophylpink) to the market.

Recently a demand for natural products - and rather heavy complaints against synthetic Cantaxanthin - has increased the interest of producing Astaxanthin in a natural process and the publications from E.A. Johnson - by producing natural Astaxanthin by fermenting a red yeast, Phaffia Rhodozyma - have been found of great interest.

This concept has now been developed into an industrial production and Grindsted Products will in 1988 be able to market Astaxanthin of natural origin.

Conc: 100 to 2000 ppm

FEEDING, DRINKING AND DIGESTION IN ATLANTIC SALMON (*SALMO SALAR*) SMOLTS
FOLLOWING TRANSFER TO SEA WATER.

Malcolm Usher and Clive Talbot

Freshwater Fisheries Laboratory
Faskally
PITLOCHRY
PH16 5LB

Feeding is suppressed immediately after transfer of smolts to sea water and about 30 days elapsed before all of the population ingested food. Changes in plasma ions indicate that hydromineral balance was established in 7-9 days post-transfer. All freshwater control fish fed throughout the experimental period. Recruitment to the feeding fraction of the population was independent of fish size and the median meal size did not differ when measured 7 days (33% feeding) or 30 days (96% feeding) after transfer to SW. Meal size and evacuation rate in feeding SW fish was not different from FW fish, however, the assimilation of nitrogen was 10% less efficient in SW fish. Growth of salmon in SW was significantly less than that of the FW control fish for the first 50 days following SW transfer. Eighty days after transfer the mean weight of SW fish exceeded that of the FW control population.

Gut pH and ionic content of digesta indicated distinct differences between the luminal milieu in SW and FW fish. Stomach pH was not significantly different between FW and SW fish but the pH of the mid- and hind-gut of SW fish is more alkaline. No significant differences were observed between the proteolytic activities of mid-gut homogenates from SW and FW fish sampled 7 and 29 days post-transfer. Proteolytic activity was not affected in either SW or FW fish by the presence of Mg^{2+} ions (150 mM.l^{-1}) or sulphate ions (70 mM.l^{-1}). Enzyme pH profiles were similar for mid-gut homogenates from both SW and FW fish with peak activity occurring at pH 9.2.

Drinking commenced within a few hours following transfer to SW, gradually increasing to a plateau after about 100h. The drinking response appears to follow changes in plasma osmolarity. Water content of digesta in the stomach of SW fish is higher than in FW fish but there were no differences in digesta water content in the mid- and hind-gut. Drinking rate of around $7.5 \text{ ml.kg}^{-1}.\text{h}^{-1}$ was unaffected by the amount of food in the stomach.



Photoperiod and Temperature Effects on Early Development and
Reproductive Investment in Atlantic salmon (Salmo salar L.)

John E. Thorpe and Colin E. Adams* (Poster)

Freshwater Fisheries Lab. Pitlochry, PH16 5LB, Scotland

(Address for communication)

*Zoology Department, University of Glasgow, Glasgow, G12 8QQ
Scotland

Early studies attempting to relate general parental development rates with reproductive investment, tended to emphasise the importance of the marine phase of the salmon's life-cycle, when examining the relationships between developmental rates (as estimated by sea-age) and fecundity (egg numbers) or egg size, largely ignoring the freshwater phase as unimportant. It is now generally accepted that there is a high degree of variability in parr maturation and smoltification rates, both between and within populations and families. This has been shown to be a function of overall growth and developmental rates of parr, and is subject to genetic and environmental influences on the population. One recent investigation has shown that parental river age, used as an indicator of developmental rate, gives a better indicator of fecundity and egg size, than sea age.

Current work has shown that, within a group of sibling Atlantic salmon parr, it is possible to manipulate developmental rate environmentally. This is achieved by controlling "growth opportunity" via photoperiod (which influences opportunity to feed) and temperature manipulation (which influences opportunity to feed, feeding rate and assimilation efficiency).

Data are presented to show that increased "thermal sum" ($^{\circ}\text{C}$ hours daylight) used as an index of growth opportunity, effectively increases reproductive investment and that this is associated with an overall increase in general developmental rate.

EFFECTS OF PROLONGED EXERCISE ON GROWTH AND BODY COMPOSITION OF
FIRST-FEEDING FRY OF ARCTIC CHARR (*Salvelinus alpinus* L.)

Jørgen Schou Christiansen and Malcolm Jobling

Institute of Fisheries, University of Tromsø, P. O. Box 3083
Guleng, N-9001 Tromsø, Norway

Several studies indicate that prolonged moderate exercise of salmonids leads to hypertrophy of muscle cells and improvement of growth, stamina and survival.

The following experiment was carried out in order to examine behaviour, growth and changes in body composition of moderately exercised Arctic charr.

First-feeding fry (size at start: 0.13 mg; 25-30 mm) were divided into "trained" and "untrained" groups (3 replicate tanks per group).

"Trained" fry were exercised at a constant water velocity of 4-6 cm per second in circular tanks.

A grid placed in the bottom of each tank enabled fry to rest. Water exchange rates were identical for all tanks and temperatures ranged from 12.6 C (Aug.) to 0.2 C (Dec.). The same amount of feed was offered to both "trained" and "untrained" fish during the 190 day experiment. There was no mortality in either group.

There were marked differences in behaviour between "trained" and "untrained" fish. Fry in the "untrained" group remained more or less inactive on the grid for most of the time but during feeding they swam to and fro taking feed from both the bottom and surface. "Trained" fry displayed positive rheotaxis and distributed themselves evenly in the whole water column. Feeding took place only at the surface. At any given time only small numbers of fry were observed resting on the grid.

At the end of the experiment the weight of "trained" fry (1.42 g) exceeded that of fry reared in still water (1.20 g) by approximately 20 %.

Analyses of body composition (dry matter, total lipid, protein, ash and total energy) were carried out for samples of "trained" and "untrained" fish taken at the start, at the midpoint and at the end of the experiment. In addition analyses of lipid classes were carried out in order to investigate neutral lipids/polar lipids and PUFA/SFA composition.

LABORATORY STUDIES ON THE USE OF ROTIFERS (Brachionus plicatilis
O.F. MÜLLER) FOR STARTFEEDING COD LARVAE (Gadus morhua L.)

I. OPSTAD,¹ B. STRAND,² I. HUSE,¹ O. GARATUN-TJELDSTØ³

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

² A/S Austevoll Fiskeindustri, N-5392 Storebø

³ Biochemical Institute, University of Bergen, N-5000 Bergen Norway.

Up untill today we have to use live feed to get cod larvae beyond metamorphosis.

Rotifers (Brachionus plicatilis) enriched with different types of feed at different feeding times have been tried out to startfeed cod larvae. The rotifers fed with a combination of fishmeal and cod roe for more than 4 hours gave the best growth. The temperature played an important role on the growth rate.

Chemical analysis have shown that rotifers enriched with fishmeal and cod roe had high content of highly unsaturated fatty acids and amino acids, specially methionine and cyctein (Olsen et al., 1985).

FISH ROE AS MAJOR COMPONENT IN START-FEED FOR MARINE FISH LARVAE

Oddvard Garatun-Tjeldstø*, Ingegjerd Opstad^S,
Tom Hansen^E and Ingvar Huse^S

* O.Garatun-Tjeldstø, Department of Biochemistry, University of Bergen, Årsadveien 19, N-5000, Bergen, Norway.
^S I. Opstad and I.Huse, Austevoll Marine Aquaculture Station, N-5392 Storebø, Norway.
^E T.Hansen, Matre Aquaculture Station, N-5198 Matredal Norway.

Marine fish larvae are, with few exceptions, dependent upon the supply of live feed for growth and development during the start feeding period. Attempts have been made to substitute live feed with artificial feed, based upon fish roe(1), dried zooplankton, fish meals, hens-egg, and several types of commercial products.

Cod larvae (*Gadus Morhua*) were start-fed in laboratory tanks using fish roe based diets. Although metamorphosis did not occur, due to a high mortality rate, it is concluded that roe based feeds supplied with peptone addition, and coated with rigid fat or stearyl, are capable of initiating development of the gut, filling of the swim bladder, and producing larval growth (2).

Prolonged survival and growth of cod larvae was obtained in start-feeding experiments with dry feeds, based upon the roe from: cod, herring, capelin, torske, ling, blue ling, and egg-yolk from ~~spurdog~~ and salmon. The two latter materials were also given as start-feed to halibut larvae (Suggested by J.V.Helvik*), and gave prolonged survival.

Most marine fish larvae are too small to ingest whole roe particles, and in their case the roe must be ground and re-particulated by rigid fat, stearyl or binders. Salmon larvae were large enough to ingest whole roe particles. This gave a growth response comparable to the best commercial feeds available. Grinding and reparticulation resulted in a lower growth response, which may indicate that improvements can be obtained by adjusting the particle properties (coat, leakage, hygiene.)

The work is supported by NFFR contract I.108.02 and V.108.010, and by Feitsildfiskernes salgslag.

References: 1) G.Molvik et al., 1984. Properties of a new diet for fish larvae, including cod (*Gadus Morhua* L) - In E. Dahl et al., (ed) Flødevigen Rapportserie. 1: ISSN 0333-2594 pp.203-211.
2) O.Garatun-Tjeldstø, 1986. Startfôr- en flaskehals i marint oppdrett. Norsk fiskeoppdrett 11. pp 49-50 and 12. pp 15-17.

EXPERIENCES WITH WASTEWATER-CULTURED Daphnia IN THE
STARTFEEDING OF RAINBOW TROUT

Göran Dave

Department of Zoophysiology, University of Göteborg,
Box 250 59, 400 31 Göteborg, Sweden

Water fleas (Daphnia magna) were cultured in three 1.2 m³ tanks fed with treated sewage water (activated sludge without tertiary chemical treatment) from February to May 1986. Concomitantly with an increase in temperature from 8 to 18°C the density in the tanks increased from 30 water fleas per litre to 1000 water fleas per litre, which corresponds to an increase in biomass from 1.4 to 190 mg/l, expressed as dry weight. During the second half of the experiment ninety rainbow trout (Salmo gairdneri) fry (0.15 g) were stocked in net pens within the tanks. After 56 days they had reached a mean weight between 1.1 and 2.4 g (4 groups), which corresponds with a daily growth rate of 3.7 to 5.1 %, with a survival of 95 %. Thus, the results have shown that the nutrients in domestic sewage water can be utilized with a fairly simple technique for production of fish for stocking purposes, on the condition that the addition of toxic compounds is within acceptable limits.

FEED INTAKE, DIGESTIBILITY AND BRAIN NEUROTRANSMITTERS IN RAINBOW TROUT UNDER HYPOXIC CONDITIONS.

POULIOT, T., J. de la NOUË and A.G. Roberge

Centre de recherche en nutrition, Université Laval,
1312 Pav. Comtois, Ste-Foy (Québec), Canada G1K 7P4

Brain concentration of the neurotransmitters serotonin (5-HT) and catecholamines depends upon the availability in the plasma of their precursors, tryptophan and tyrosine, respectively. This plasma concentration reflects the dietary supply and is linked to the quality, i.e. amino acid profile, of dietary protein.

An increased resistance to stress shows up when the organism succeeds in maintaining the initial equilibrium between 5-HT and norepinephrine (NE) in the central nervous system. Our aim was therefore to influence the response of rainbow trout to hypoxia (60% oxygen-saturated water) by modifying the dietary protein quality. We therefore used diets of similar composition (isoproteic, isoenergetic) to that of a commercial feed; the sources of protein (animal, vegetal) were different, however.

The results for the three diets show that experimental hypoxia did not change the apparent digestibility of protein (total nitrogen), total energy and dry matter. There was, however a slightly better amino acid assimilation in the case of the animal and commercial diets. Moreover, the type of protein (animal or vegetal) of the diet did influence the concentration of 5-HT, dopamine (DA), NE and 5-hydroxyindolacetic acid (5-HIAA) in the hypothalamus, mid-brain and fore-brain of rainbow trout after a 149-day adaptation period to the diets; the final concentrations measured after hypoxia stress were also different according to the diet.

After the hypoxia stress, the fish fed the animal diet maintained the pre-stress equilibrium between the serotonergic and catecholaminergic pathways. Moreover, these trout showed a feed intake which remained normal or came back to normal after stress whereas the fish receiving the other diets did not. These results are in agreement with our hypothesis.

A poor resistance to stress, especially to hypoxia, in aquaculture is responsible for reduced growth and increased sensitivity to infections. The use of "special" diets, with appropriate amino acid profiles, could be an interesting innovation for fish husbandry.

(Supported by FCAR Funds (Québec) and CRSAQ Grant).

INCREASING IMMUNOLOGICAL RESISTANCE BY HIGHER MUSCLE ACTIVITY IN
PELAGIC FISHES BY A MOVING FOOD SUPPLY:

Jens W. Jebsen

Institutt of nutrition, Directorate of Fisheries,
P.O.Box 4258
N-5028 Bergen

Cold-blooded fishes have, besides a simplified phagocytosis, some general form of protection against bacteria and parasites. Here, probably, belongs the enzymatic formation of formaldehyde (FA) from trimethylamine-oxide. As the production of antigens below 10°C is low, but the development of FA is at maximum 3°C . This protection is important in cold areas. The formation of FA is high in the (less active) gadoid fishes, about 50 mg FA/100g in stored, sterile muscle. However, in kidney and gall-bladder there is about 1000 times higher activity.

In pelagic fishes we found low enzymatic FA formation, However a low concentration of hemo-proteins, (down to 0.5g / ml) strongly activated the enzyme. Therefore the formation of FA will increase with the increasing blood circulation caused by higher muscle activity. This maybe obtained by accustoming the fishes to swim after a moving food supply.

THE APPEARANCE OF SOLUBLE ANTIGEN, HORSERADISH PEROXIDASE (HRP), IN
TISSUES OF THE RAINBOW TROUT (SALMO GAIARDNERI, RICHARDSON), SUBSEQUENT
TO VARIABLE DIP-IMMERSION PROCEDURES.

E. McLEAN, R. ASH & P.A.B. WESTCOTT

School of Biomedical Sciences
University of Bradford
Richmond Road
Bradford BD7 1DP
W. Yorks. UK

Direct immersion is the most frequently used method of fish vaccination. The advantages of this technique are many, but perhaps most importantly, it permits the delivery of vaccines to large numbers of fish, of variable age and size, with the minimum amount of handling and hence stress. The efficacy of applied antigens under such procedures is dependent upon the immunogenicity of the antigen, the dose administered and the immersion time. Additionally, the effectiveness of vaccination may also depend upon the dietary and immunological status of the fish and may be further influenced by season. Currently, little information exists upon the quantitative absorption and subsequent tissue localisation of antigens post-immersion. Thus, a series of experiments were designed to determine tissue localisation of antigen after variable dip-immersion procedures. Additionally, the time-course of blood and tissue accumulation of horseradish peroxidase was assessed. Results from these studies revealed that the absorption of the antigenic protein macromolecule horseradish peroxidase by juvenile rainbow trout was independent of immersion time and that considerable variation in tissue accumulation and clearance occurred.

The importance of these findings, with respect to the commercial application of dip-immersion vaccination upon the fish farm are discussed, whilst comparisons of horseradish peroxidase accumulation within specific tissues of the rainbow trout are made with orally administered animals.

EFFICIENCY OF VITELLOGENIN SYNTHESIS IN ATLANTIC SALMON (SALMO SALAR)
AT DIFFERENT ACCLIMATION TEMPERATURES

Olin, Thomas and von der Decken, Alexandra

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

During vitellogenin synthesis in liver the requirement of body nutrients increases. To learn more about the effect of acclimation and incubation temperatures vitellogenin synthesis was studied in immature salmon (Salmo salar). The fish were acclimated at 8°C or 16°C and injected with 17- β -estradiol, 5 mg/kg body weight once a week for two weeks. Protein synthesis *in vitro* was determined at 8°C and 16°C in the postmitochondrial supernatants of liver homogenates (Olin, T. and von der Decken, A., *Physiol. Zool.*, in press, 1987). Newly smoltified fish were acclimated at 8°C. Protein synthesis measured at 8°C increased 5.7-fold in the induced as compared to the control liver. At 16°C protein synthesis increased 2.5-fold. In presmolt salmon acclimated at 16°C the increase in protein synthesis was 5.9-fold at the incubation temperature of 8°C and 2.7-fold at the incubation temperature of 16°C. The relative increase in protein synthesis between 8°C and 16°C was significantly higher in the control than the induced liver. This accounted for the differences in effects observed between control and induced fish at the two incubation temperatures. The efficiency of vitellogenin synthesis was expressed as the ratio of induced protein synthesis in liver over the vitellogenin content per ml of serum. At the acclimation temperature of 8°C smolt showed a ratio of 15.0 and at the acclimation temperature of 16°C presmolt showed a ratio of 3.1. The conditions for smolt acclimated at 16°C are currently under investigation. The results indicate a more efficient vitellogenin synthesis and/or export from the liver to the blood at 16°C than at 8°C.

Support: The Swedish Council for Forestry and Agriculture Research, Project No. 520/85 DV38 and The Swedish Natural Science Research Council, Project No. B-Bu 2520-111.

Physiological changes in muscle of rainbow trout with different feed levels

Kiessling, A., Kiessling, K.-H., *Storebakken, T. and Johansson, L.

Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, P.O. Box 7046, S-750 07 Uppsala, Sweden

*Institute of Aquaculture Research, N-6600 Sunndalsöra, Norway

A problem in practical fish farming is to administrate adequate feed levels. Overfeeding is an economical and environmental problem, whereas too low feed levels will lead to decreased growth and possible harmful effects of the fish.

To study the physiological effects of underfeeding on rainbow trout an experiment was set up at AKVAFORSKs research station in Sunndalsöra, Norway. The fish were fed from startfeeding either adequate level (Storebakken 1986 PhD-Thesis) or 50 and 25% of that level. To study the possible recovery capacity the groups were split after the first growth period and their feed level were changed (adequate level to 50 and 25% and vice versa). Samples were taken of the white and red muscle and the main fat deposits one and eight months after splitting, to enable studies of short and long time effects. Fishes for sampling were selected, on weight basis, from the distribution mean. The experiment is now continued in the sea.

The physiological parameters analysed are enzyme activities, histochemical staining and levels of protein, glycogen, fat and DNA. The fatty acid spectra are also analysed (see Kiessling, Johansson, Storebakken Poster abstract). This parameter selection is based on earlier studies of starved fish (Kiessling, A., Kiessling, K.-H., in prep. and Kiessling, A. et al. in prep.). The growth of the fish showed a rapid response to changed feed level, while only citratesynthetase showed any effect on 50% level after one month. Glycolysis, beta-oxidation and cytochrome oxidase showed a decrease in the 25% level. The protein content was rapidly affected when fishes were moved between different levels.

PREPARATION AND CHARACTERIZATION OF CHROMATIN PROTEINS FROM SALMON
(SALMO SALAR) LIVER

Waters, S. and von der Decken, A.

The Wenner-Gren Institute for Experimental Biology,
University of Stockholm, Biology F 3, S-106 91 Stockholm, Sweden

During maturation of salmon the synthesis of vitellogenin in liver is markedly enhanced. The protein is transported in the blood to the gonads and takes part in the formation of yolk proteins. Activation of the vitellogenin genes in liver is the initial step in the enhancement of vitellogenin synthesis. An alteration in the amount of chromatin proteins bound to DNA together with their internal distribution may be responsible for the accessibility of the genes to transcription. To learn more about chromatin proteins and their function in gene activation a preparation procedure was worked out to separate chromatin proteins from liver of nonmature salmon.

Nuclei were isolated from liver and incubated with micrococcus nuclease to separate the chromatin into regions of low and high transcription activity. The chromatin fractions were extracted stepwise with NaCl.

A problem encountered when studying nuclear proteins is that only histones are present in such amounts that they are easily obtainable. An accurate method for separation and quantification of the less abundant proteins is required. Many proteins are closely related with respect to molecular weight and charge and separating them demands high resolution from the method applied. Most of these obstacles were overcome by using anion exchange high performance liquid chromatography (HPLC). As an additional method of protein separation polyacrylamide gel electrophoresis in SDS was used. Chromatin proteins of the high mobility group (HMG) were extracted separately from salmon liver. They served to identify their counterparts in the protein pattern obtained by HPLC or polyacrylamide gel electrophoresis. The combined methods of salt extraction, HPLC and polyacrylamide gel electrophoresis will be used in further experiments to study chromatin proteins of importance to vitellogenesis.

Support: The Swedish Council for Forestry and Agriculture Research, Project No. 520/85 DV38 and The Swedish Natural Science Research Council, Project No. 8.-Bu 2520-111.

A Contribution to the energy metabolism of tilapia (*O. niloticus*).

K.O. Günther, M.F. Osman and K. Meyer-Burgdorff

Department of Animal Physiology and Nutrition, University of Goettingen, Oskar-Kellner-Weg 6, D-3400 Goettingen

The energy metabolism of fish must be assessed in the same way as for terrestrial animals. The determination of the partition of food energy (GE) into metabolisable energy (ME) and energy retention (NE_g) is therefore of importance. The measurement of gross energy intake and of net energy retention are carried out using a bomb calorimeter or following the comparative slaughter technique respectively. Difficulties arise from measuring energy excretions of fish in order to estimate the metabolisable energy. The heat production can be determined from the oxygen consumption of fish following the principles of indirect calorimetry. In combination with the comparative slaughter technique it is possible to calculate the metabolisable energy of a food ratio.

A series of experiments was carried out with *O. niloticus* kept in a recirculating system at 26° C water temperature under different feeding regimes. Under the experimental conditions maintenance requirement amounted to 55 kJ ME kg^{0.8}. In relation to feed intake energy metabolism for growth ranged between 150 kJ ME kg^{0.8} and 210 kJ ME kg^{0.8} per day at feed intake of 14 or 24 g kg^{0.8} d respectively. Independent of feed intake the utilization of ME above maintenance for growth was about $k_g = 0.65$. The retained energy consisted of 62% protein, 35% lipids and 3% carbohydrate - again independent of feed intake. The efficiency of utilizing ME for protein retention in growing Tilapia was estimated at $k_p = 0.60$.

INFLUENCE OF SOYABEAN MEAL AND SQUILLA MEAL BASED DIETS
ENRICHED WITH SARDINE OIL ON THE GROWTH AND ORGANOLEPTIC
QUALITY OF COMMON CARP, CYPRINUS CARPIO (LINN.)

M.C.Nandeesh, N. Basavaraja, P. Keshavanath, T.J. Varghese
and H.P.C. Shetty.

College of Fisheries, University of Agricultural Sciences,
Mangalore-575 002, INDIA.

The use of nutritionally balanced feeds in carp culture is gaining popularity in India in recent years. Though fish meal incorporated diets result in good fish growth, the escalating cost and uncertainty of availability of fish meal have necessitated the search for other alternative protein sources. Earlier experiments conducted at the College of Fisheries, Mangalore substituting fish meal in the compounded feed with soyabean or squilla meal did not yield satisfactory results. As an extension of this study, a field trial was conducted over a period of 98 days to evaluate the merits of five new feeds formulated using different combinations of soyabean meal, squilla meal, fish meal and sardine oil enrichment vis-a-vis fish meal based standard diet on the growth and organoleptic quality of common carp. Of the five treatments tried, soyabean-squilla combination enriched with 5% oil consistently showed higher growth and proved to be the best, yielding an increase of 24.99% body weight over that obtained with the fish meal based control diet. The final fish weights obtained with soya-fish meal combination and soyabean meal enriched with 8% sardine oil were lower by 8.16% and 4.82% respectively than that obtained with the control diet.

Organoleptic evaluation showed marked variation in various attributes like colour and gloss of skin and colour of raw flesh and odour in cooked flesh.

The results indicate the possibility of using squilla meal, reported to be rich in lysine, at a reduced level in combination with soyabean meal, known to be deficient in lysine and methionine, as an economic substitute for fish meal in carp diet.

THE EFFECT OF 17α -METHYLTESTOSTERONE ON THE GROWTH AND FOOD CONVERSION OF MAJOR CARPS

Diprangshu Deb and T.J Varghese

Dept. of Aquaculture, University of Agricultural Sciences
College of Fisheries, Mangalore- 575002, INDIA.

Fingerlings of catla, rohu, mrigal and common carp were fed pelleted feeds incorporated with 17α -methyltestosterone (MT) at concentrations of 0, 1, 3, and 5 mg/kg (ppm) diet for 98 days. The experiment was conducted in 12 cement cisterns of 25 m² each. Each cistern was stocked with five uniform sized fingerlings of each species at a total stocking density of 8000 fingerlings/ha. The results of the experiment revealed that while catla, rohu and common carp grew better in response to 1 ppm MT, the response of mrigal was better to 3 ppm of the hormone. The difference in growth between various treatments was statistically significant, except between control and 5 ppm treatment. Fishes receiving 1 ppm MT showed higher food conversion efficiency than those receiving either 3 or 5 ppm of the hormone. The increase in fish production in 1 ppm MT treatment was 63.85% more than the control group.

Mrigal 3 ppm 17α -MTH
Rohu }
Catla } 1 ppm
Carpio }