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FISH SILAGE IN AQUACULTURE DIETS

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ABSTRACT

Fish silage was made from ground, whole Pacific whiting by using 2% sulfuric acid and 0.75% propionic acid by weight as the acidulent. After 3, 6, and 20 days, silage was removed, neutralized with calcium hydroxide, and drum-dried. Two additional silage products were made from unneutralized 20-day silage and silage neutralized with sodium hydroxide. Vacuum-dried Pacific whiting meal was used in the control diet. Dry pelleted diets were made using the Abernathy diet formulation S8-1, modified by replacing the fish meal with either drum-dried silage or vacuum-dried Pacific whiting meal.

After 12 weeks, no significant differences were observed in growth or feed conversion between rainbow trout fed either the control diet or diets containing 3- or 6-day-old silage. Growth and feed conversion were significantly affected by feeding diets containing silage that had been allowed to hydrolyze for 20 days. No differences in growth were observed between groups of trout fed diets containing 20-day unneutralized silage, or silage neutralized with calcium hydroxide or sodium hydroxide. Acidification of the control diet significantly affected growth and feed conversion. Neutralization of the acidified control diet with calcium hydroxide restored growth and feed conversion.

INTRODUCTION

The development and expansion of the fishery within the 200-mile zone along the coastal United States has the potential to greatly expand domestic groundfish harvest and processing. Waste (fillet scrap, undersized fish, and by-catch) that may be generated represents a disposal problem to the fishing industry, but, at the same time, a potential resource to the animal feed industry. Processing this waste into fish meal may be difficult because the supply is highly variable and fish meal plants require a relatively constant supply of raw material to operate economically. Ensilaging is an inexpensive way to store the raw material until it can be processed into a useful by-product.

Fish silage is fish waste or whole fish liquefied by the action of

added or endogenous proteolytic enzymes in the presence of added acid. Fish silage can be stored for long periods without refrigeration or freezing because the low pH of the liquid prevents bacterial spoilage. Mold growth is prevented by the use of propionic acid or formic acid as the total or partial acidulent. Acid preservation of plant material was developed in Finland in the 1920's and fish silage has been prepared in commercial quantities in northern Europe since 1948 (Tatterson and Windsor 1974).

Fish silage has been used in fish feeds with varying success depending upon the species of fish, the type of acid used in the ensiling process, and the method of processing. In Norway, feeding trials have shown that Atlantic salmon are more sensitive to the type of acid used than are rainbow trout (Asgard and Austreng 1981). Formic acid (2.2% w/w) or sulfuric acid (2.5% w/w) in combination with formic acid (1.1% w/w) have given the best results with Atlantic salmon. Rungruangsak and Utne (1981) fed diets acidified with 2.5% (w/w) hydrochloric, formic, or sulfuric acid to rainbow trout for 140 days and measured growth and proteolytic activity in the digestive tract. Hydrochloric acid did not affect growth or proteolytic activity while formic acid depressed both. The addition of sulfuric acid reduced growth but did not reduce proteolytic activity in the stomach. In both of these studies moist diets were fed to the fish. In the United States, moist diets are fed to Pacific salmon fingerlings, while dry diets are fed to trout, catfish, and, in some hatcheries, to salmon. Fish silage probably does not have any nutritional or economic advantage over the wet fish portion of moist diets. The wet fish portion comprises 30% of moist salmon diets and is made from a combination of fish viscera and scrap fish. In addition there is no need to produce silage for this purpose since there should be an abundant supply of viscera and scrap fish now and in the foreseeable future. On the other hand, dried protein supplements of marine origin are used in many dry aquaculture diets and demand for these products should remain high.

Dried fish silage has not been directly compared to fish meal in feeding trials with salmonids. Therefore this study was conducted to evaluate the nutritional value of dried silage products as ingredients in rainbow trout diets. All of the fish meal in the diets was replaced with silage to better evlauate the effects of the dietary treatments. The parameters examined were the degree of liquefaction of the silage, and neutralization of the silage with different bases.

MATERIALS AND METHODS

Groups of 50 rainbow trout (mean weight 12 g) were randomly distributed into 24 circular polypropylene, 130-liter tanks. Each tank was aerated and supplied with 2.4 liters/min of constant temperature (15°C) dechlorinated city water. Three replicate groups were fed each of the 8 experimental diets. The average weight of the fish in each tank was determined at the start of the experiment and every 28 days thereafter for 84 days.

Dry diets were made using the Abernathy diet formulation S*-1, modified by replacing the fish meal in the formulation with either drum-dried silage or vacuum-dried fish meal (Table 1). The fish silage was made by adding 2% sulfuric acid and 0.75% propionic acid (w/w) to ground, whole

Pacific whiting. The mixture was stored in a covered polypropylene container at ambient temperature (12-15°C) for 20 days and mixed daily by hand with a wooden paddle. The degree of liquefaction of the silage was determined by calculating the ratio of nonprotein nitrogen to total Kjeldahl nitrogen in the silage. Nonprotein nitrogen was measured by mixing a sample of silage with 2 times volume of 20% trichloroacetic acid. After filtration, Kjeldahl nitrogen was determined on the filtrate (Raa and Gildberg 1976). After 3, 6, and 20 days, a portion of the mixed silage was removed, neutralized with 1.6% $Ca(OH)_2$, and dried on a chrome-plated, double-drum dryer using 60 psi steam (140°C), and rotating at 2 rpm, exposing the wet silage to heat for approximately 15 seconds. Two additional portions were drum-dried after 20 days. One batch was neutralized with 1.73% NaOH before drum drying and the other batch was drum-dried without being neutralized.

Table 1. Composition of Experimental Diets

Treatment	Vacuum-dried hake meal (%)	Drum-dried hake silage (%)	Other ingredients (%)
1 - Fish meal	50		50
2 - Fish meal (acidified)	50		50
3 - Fish meal (acidified, then neutralized)	50		50
4 - 3-day silage + $Ca(OH)_2$		50	50
5 - 6-day silage + $Ca(OH)_2$		50	50
6 - 20-day silage + $Ca(OH)_2$		50	50
7 - 20-day silage + NaOH		50	50
8 - 20-day silage - unneutralized		50	50

Other ingredients include (%):

Dried whey, 10; Crab meal, 5; Wheat middlings, 13.9; Blood flour, 5; Brewer's yeast, 5; Vitamin premix, 1.5[a]; Trace mineral premix, 0.1[b]; Choline chloride, 0.5; Soybean oil, 9.

[a]Vitamin premix contains the following per kg of premix: 7.04 g D-calcium pantothenate; 2.06 g pyridoxine-HCl; 3.52 g riboflavin; 146.7 g niacin-amide; 0.85 g folic acid; 2.86 g tiamin mononitrate; 39.6 mg biotin; 3.96 mg vitamin B_{12}; 737 mg menadione sodium bisulfate; 33.4 g alpha tocopheryl acetate; 29.34 I.U. vitamin A palmitate or acetate; 17.6 g myo-inositol; and 59.4 g ascorbic acid.

[b]Mineral premix contains the following per kg of premix: 184.8 g $ZnSO_4$; 206.8 g $MnSO_4$; 49.5 g $FeSO_4 \cdot 7H_2O$; 3.85 g $CuSO_4$; and 0.836 g KIO_3.

The Pacific whiting meal used in the control diets was made from the same batch of ground fish from which the silage was made. The ground Pacific whiting was vacuum-dried at 584-635 mm of mercury in a steam-jacketed dryer. Portions of this material were acidified and dried with 2% sulfuric acid and 0.75% propionic acid (w/w), or with 2% sulfuric acid, 0.75% propionic acid, and 1.6% calcium hydroxide (w/w).

The amount of feed supplied to the fish was determined by the

method of Butterbaugh and Willoughby (1967) using a hatchery constant of 16. The trout were hand-fed 3 times per day. Each week the fish received 7 days rations in 5 days. Samples of each diet and of 10 fish from each treatment were taken at the end of the experiment for determination of proximate and mineral composition. Proximate analysis was determined using conventional procedures (AOAC 1975). Samples of the ashed material were prepared for mineral analysis by dissolving the ash in 1 ml nitric acid and 1 ml hydrochloric acid, diluting to 20 ml with deionized water, filtering, and diluting to 200 ml. The samples were analyzed for mineral concentration using an inductively-coupled, Argon plasma spectrophotometer (Jarrell-Ash AtomComp, Fisher Scientific Company, Waltham, Mass.).

Daily instantaneous growth rates, food conversion values, and protein efficiency ratios (PER) were calculated for each dietary treatment for the 84-day experimental period, except for diet 4. An insufficient amount of feed for diet 4 remained to continue feeding longer than 63 days. Analysis of variance was performed to test for significance among treatment means for daily instantaneous growth rates, feed conversion values, and mean final weights (Steel and Torrie 1960). Duncan's New Multiple Range Test was used to compare treatment mean values.

RESULTS

Neutralization of the silage with calcium hydroxide or sodium hydroxide greatly increased the amount of calcium or sodium and therefore the percent of ash in the final diets (Tables 2 and 3). Diets 3-6 contained approximately 5% calcium, compared to 3-3.5% in the other diets. Diet 7, made with silage neutralized with sodium hydroxide, contained 2.7% sodium, while each of the diets contained less than 1%.

Table 2. Proximate Composition of the Experimental Diets

Diet	Treatment	% Moisture	% Protein	% Fat	% Ash
1	Fish meal	7.6	40.4	13.0	12.2
2	Fish meal (acidified)	7.8	41.0	12.5	12.0
3	Fish meal (acidified, then neutralized)	7.0	41.5	13.4	15.2
4	3-day silage + $Ca(OH)_2$	7.8	42.0	12.7	16.4
5	6-day silage + $Ca(OH)_2$	7.3	42.4	13.0	16.9
6	20-day silage + $Ca(OH)_2$	6.7	43.2	13.3	15.4
7	20-day silage + NaOH	7.0	42.8	13.0	17.2
8	20-day silage	7.5	41.4	12.3	12.3

The final weight, daily instantaneous growth rate, and feed conversion values of the groups of fish were significantly affected by dietary treatment (Table 4). Acidification of the control diet by the addition of sulfuric acid (diet 2) significantly reduced final weight of the fish ($P < 0.05$) compared to the control group. Daily instantaneous growth rate, feed conversion values, and protein efficiency ratios were also

affected. The addition of calcium hydroxide to the acidified control diet restored these values to levels equal to the control group.

Table 3. Mineral Composition (ppm) of the Experimental Diets

Element	Dietary treatment							
	D1	D2	D3	D4	D5	D6	D7	D8
Al	130	14	127	126	130	100	113	106
Ca	35000	30100	47300	50300	52300	49900	33900	33600
Co	1.41	0.79	1.36	1.28	0.99	0.86	0.59	0.89
Cr	6.49	3.09	2.82	11.0	12.7	8.3	3.1	2.8
Cu	9.5	10.5	11.8	7.6	11.0	7.3	5.4	10.5
Fe	432	461	442	280	477	275	274	286
K	10500	11400	11000	11900	11200	11900	11200	12400
Mg	2320	2190	2220	2300	2200	2110	2230	2330
Mn	86	93	134	68	95	88	68	99
Na	8610	7020	7430	8280	8110	7400	7360	8310
P	17200	19400	19100	16200	16300	16400	17000	16900
Sr	204	181	216	217	218	203	189	189
Zn	174	164	158	107	143	119	133	195

Table 4. Final Weight, Daily Instantaneous Growth Rate, Food Conversion Values, and Protein Efficiency Ratios of Rainbow Trout after 84 Days of Feeding (initial weight = 12 g)

Treatment	Final weight[a]	Daily instantaneous growth rate[a]	Food fed/ wt gain[a]	Protein efficiency ratio[b]
1 - Fish meal	58.4AB	0.0191AB	1.21A	1.89
2 - Fish meal (acidified)	51.9D	0.0175CD	1.36B	1.65
3 - Fish meal (acidified, then neutralized)	56.9ABC	0.0185ABC	1.25AB	1.85
4 - 3-day silage + $Ca(OH)_2$[c]	---	0.0192AB	1.34B	---
5 - 6-day silage + $Ca(OH)_2$	59.9A	0.0196A	1.27AB	1.72
6 - 20-day silage + $Ca(OH)_2$	53.5CD	0.0175CD	1.37B	1.57
7 - 20-day silage + NaOH	50.7D	0.0170CD	1.50C	1.45
8 - 20-day silage	49.5D	0.0166D	1.50C	1.49

[a]Means followed by the same letter are not significantly different ($P > 0.05$).

[b]Protein efficiency ratio = weight gain (g)/protein fed (g).

[c]Treatment was discontinued after 9 weeks. Daily instantaneous growth rates and food fed/weight gain values are for the period 0-9 weeks.

The degree of silage liquefaction before it was dried and incorporated into the diets also affected the performance of fish in the treatment groups. The ratio of nonprotein nitrogen to total nitrogen in the silage was 41% at the start of the liquefaction period, 50% after 3 days, 59% after 6 days, and 79% after 20 days of storage. The final weight of the fish fed the 20-day silage was significantly lower ($P < 0.05$) than

the final weight of fish fed 6-day silage. Daily instantaneous growth rates were also reduced. Feed conversion values were not significantly different for fish fed diets containing silage at different stages of liquefaction and neutralized in the same way (diets 4, 5, and 6). The protein efficiency ratio of fish fed diet 5 was higher than that of fish fed diet 6.

Fish fed diets containing 20-day silage unneutralized (diet 8) or neutralized with either calcium hydroxide (diet 6) or sodium hydroxide (diet 7) had statistically similar final weights and daily instantaneous growth rates ($P > 0.05$). The feed conversion values of fish fed diet 6 were significantly lower ($P < 0.05$) than were those of fish fed diets 7 and 8. Protein efficiency ratios of fish fed diets 6-8 showed the same trend as the feed conversion values.

The proximate composition of the fish at the end of the experiment showed no treatment effect (data not shown). No differences in the whole body mineral concentrations of the fish from each dietary treatment at the end of the experiment were found (Table 5).

Table 5. Whole Body Mineral Concentrations (ppm) of Fish after 84 Days of Feeding

Element	Dietary treatment						
	1	2	3	5	6	7	8
Al	48.2	50.2	44.2	53.6	48.2	37.2	46.8
Ca	19100	22800	20100	22100	23200	22700	24400
Cr	1.1	1.7	1.1	1.9	0.7	0.9	0.9
Cu	14.3	20.2	8.3	14.5	7.2	8.9	10.1
Fe	65.7	73.8	53.7	74.7	49.7	46.0	48.6
K	9080	10500	8890	10200	10000	9530	10500
Mg	1270	1350	1260	1290	1240	1350	1340
Mn	5.1	7.1	6.0	9.1	4.3	4.0	4.9
Na	6110	5810	6040	5820	5380	5690	5730
P	16200	18400	16700	17100	18600	18500	19500
Sr	49.5	71.1	58.0	53.8	54.9	57.4	77.6
Zn	111	119	99	122	117	113	119

DISCUSSION

The acidity of the diets appeared to be partially responsible for the depression in weight gain and increase in food conversion values observed among the dietary treatments in this experiment. Rungruangsak and Utne (1981) found that the addition of 2.5% sulfuric acid (w/w) to a moist feed reduced weight gain and proteolytic activity in sections of the intestine of rainbow trout. In our experiment, the amount of sulfuric acid in the diets was higher (5.9%) since the silage was dried and comprised 50% of the diet. The lower PER value of diet 2 compared to diets 1 or 3 may have been caused by reduced proteolytic activity in the digestive tract of the trout in that dietary group. The observation that neutralization of the acidified control diet with $Ca(OH)_2$ produced final weight values, food conversion values, and PER values similar to the control groups further supports this contention.

Neutralization of silage with $Ca(OH)_2$ greatly increased the dietary calcium levels to levels that were found in practical diets that have caused cataracts in salmon. Ketola (1979) suggested that increased levels of dietary calcium impaired the absorption or utilization of dietary zinc in trout diets, resulting in the development of cataracts. Heth et al. (1966), however, showed that dietary calcium reduced absorption of dietary zinc in rats only in the presence of increased dietary phosphorus. Analysis of the results of our experiment indicates that high dietary calcium levels did not interfere with absorption of dietary zinc, since whole body zinc levels remained high and no cataracts were observed. Addition of $Ca(OH)_2$ to fish silage acidified with sulfuric acid results in the formation of $CaSO_4$, a compound that is only slightly soluble and ionizable. This property undoubtedly accounts for its lack of interference on the bioavailability of zinc.

During the process of liquefaction, intact fish proteins are hydrolyzed to small peptides and free amino acids (Raa and Gildberg 1976). The percentage of total protein in silage that can be precipitated with trichloroacetic acid decreases rapidly to approximately 20% at completion of the liquefaction process. Although free amino acids are rapidly absorbed in the intestine of fish, the growth rates of carp (Aoe et al. 1970, 1974) and coho salmon (S. Arai, personal communication, National Research Institute of Aquaculture, Mie-Ken, Japan) fed diets containing either high levels of free amino acids or protein hydrolysates are usually slower than for fish fed similar diets containing intact proteins. Plakas and Katayama (1981) found that absorption of amino acids from an amino acid diet occurred more rapidly in carp, which were reared at 25°C, than did absorption of amino acids from a casein diet, although the final apparent digestibilities of the amino acids were similar. The levels of plasma amino acids in carp fed a casein diet peaked at 4 hours after feeding while the plasma lysine and arginine levels in carp fed an amino acid diet peaked 2 hours after feeding (Plakas et al. 1980). By 4 hours, the plasma levels of lysine and arginine had returned to prefeeding levels in carp fed the amino acid diet. The peak value of plasma ammonia in carp fed the amino acid diet occurred sooner after feeding and was higher than the peak value for plasma ammonia in carp fed the casein diet. This implies a higher rate of amino acid catabolism in carp fed the amino acid diet. Yamada et al. (1981) found that peak levels of essential amino acids in plasma of rainbow trout at 10°C following a force-fed meal of amino acids occurred after 12 hours. When the plasma of trout force-fed a casein diet was examined, peak levels of essential amino acids were observed 24 hours after feeding. Thus, for rainbow trout and for carp, essential amino acid levels in the plasma peaked and returned to prefeeding levels sooner after a mean of an amino acid mixture than after a meal of casein, reducing the time available for tissue protein synthesis.

In the present study, fish fed diet 6, containing 20-day silage, had lower final weights, higher feed conversion values, and lower PER values than did fish fed diet 5, containing 6-day silage. These differences may have been caused by differences in the rate of amino acid absorption in the intestine. If the levels of amino acids in the plasma reached a peak and returned to prefeeding levels sooner in trout fed the 20-day silage diet, the amount of absorbed amino acids incorporated into tissue protein would be lower. This possibility should be verified experimentally.

It may be advantageous to stop the liquefaction process before completion by heating the silage to inactivate endogenous proteolytic enzymes. Then, further storage would not result in higher levels of free amino acids.

SUMMARY

The results of this study indicate that the length of storage of fish silage affects its nutritional value, suggesting that if silage is allowed to liquefy and is stored for a long period before being dried and used in trout diets, total replacement of fish meal by silage should not be attempted. Reducing the level of silage in the diet may limit the effects of feeding rapidly-absorbed amino acids and produce acceptable growth rates. Stopping the liquefaction process before completion by heat treatment may allow higher levels of dried fish silage to be used in the diet without affecting fish growth. In addition, this study shows that fish silage made with sulfuric acid can be neutralized with $Ca(OH)_2$ without reducing the whole body levels of zinc in the fish. Finally, this study shows that part of the reduction in nutritional value of the silage may be the result of acidification of the diet.

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LITERATURE CITED

Aoe, H., I. Masuda, I. Abe, I. Saito, T. Toyoda, and S. Kitumara. 1970. Nutrition of protein in young carp. I. Nutritive value of free amino acids. Bulletin of the Japanese Society of Scientific Fisheries 36:407-413.

Aoe, H., K. Ikeda, and T. Saito. 1974. Nutrition of protein in young carp. II. Nutritive value of protein hydrolysates. Bulletin of the Japanese Society of Scientific Fisheries 40:375-379.

Asgard, T., and E. Austreng. 1981. Fish silage for salmonids: a cheap way of utilizing waste as feed. Feedstuffs 53(27):22-24.

Association of Official Analytical Chemists. 1975. Methods of Analysis, 12th ed. Washington, D.C. 1094 pp.

Buterbaugh, G. L., and H. Willoughby. 1967. A feeding guide for brook, brown, and rainbow trout. Progressive Fish-Culturist 29:210-215.

Heth, D. A., W. M. Becker, and W. G. Hoekstra. 1966. Effect of calcium, phosphorus, and zinc on zinc-65 absorption and turnover in rats fed semipurified diets. Journal of Nutrition 88:331-337.

Ketola, H. G. 1979. Influence of dietary zinc on cataracts in rainbow trout (*Salmo gairdneri*). Journal of Nutrition 109:965-969.

Plakas, S. M., T. Katayama, Y. Tanaka, and O. Deshimaru. 1980. Changes in the levels of circulating plasma free amino acids of carp (*Cyprinus carpio*) after feeding a protein and an amino acid diet of similar composition. Aquaculture 21:307-322.

Plakas, S. M., and T. Katayama. 1981. Apparent digestibilities of amino acids from three regions of the gastrointestinal tract of carp (*Cyprinus carpio*) after ingestion of a protein and a corresponding free amino acid diet. Aquaculture 24:309-314.

Raa, J., and A. Gildberg. 1976. Autolysis and proteolytic activity of cod viscera. Journal of Food Technolgoy 11:619-628.

Rungruangsak, K., and F. Utne. 1981. Effect of different acidified wet feeds on protease activities in the digestive tract and on growth rate of rainbow trout (*Salmo gairdneri* Richardson). Aquaculture 22:67-79.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York. 481 pp.

Tatterson, I. N., and M. L. Windsor. 1974. Fish silage. Journal of Science of Food and Agriculture 25:369-379.

Yamada, S., K. L. Simpson, Y. Tanaka, and T. Katayama. 1981. Plasma amino acid changes in rainbow trout *Salmo gairdneri* force-fed casein and a corresponding amino acid mixture. Bulletin of the Japanese Society of Scientific Fisheries 47:1035-1040.