*Fisk.Dir. Skr., Ser. Ernæring, Vol. II, no. 3, s. 53–62 (1982)*

# APPARENT AVAILABILITY OF TOTAL NITROGEN, PROTEIN NITROGEN AND OF INDIVIDUAL AMINO ACIDS IN ATLANTIC COD

## *(Gadus morhua)*

By

EINAR LIED and LEIF REIN NJAA

Institute of Vitamin Research
Direktorate of Fisheries
N-5013 Nygårdstangen/Bergen, Norway

ABSTRACT

Apparent availability values of amino acid nitrogen and individual amino acids were determined by the indirect indicator method in the digestive tract of cod, which was divided into the stomach, the pyloric ceca region, the anterior, the middle and posterior ileum and the rectum. In two experiments, cod were given saithe fillet containing chromium(III)-oxide and whole sprat containing titanium(IV)-oxide. Further, cod, which had preyed on capelin were caught in the Barents Sea, and availability values were measured using calcium as an internal indicator, and the stomach contents as reference. Most of the amino acid and total nitrogen was absorbed in the anterior part of the intestine. The estimates of protein (N × 6.25) availability were biased by the content of non-protein N in the intestinal and rectal samples. Therefore the amino acid N content in feed and feces should be used for the accurate estimation of protein availability. The apparent nutritional availability values based on amino acid N were 96, 89 and 95% for cod on a mixed diet, fed whole sprat and wild cod, respectively. Corresponding values were found for single amino acids.

## INTRODUCTION

Although several reports have appeared dealing with nutrient digestibility in various fishes, little information is available on the amino acid availability. DABROWSKI and DABROWSKA (1981) examined the apparent and true digestibilities of amino acids in the gastrointestinal tract of rainbow trout (*Salmo gairdneri*) using chromium(III)-oxide as the undigestible reference substance. The fish were fed three composite diets after which contents from different gastrointestinal sections were collected and analysed for free and total amino acids. WILSON et al. (1981) measured the apparent and true digestibilities of amino acids from eleven commonly used feed ingredients for channel catfish (*Ictalurus punctatus*) using the chromium(III)-technique. The fish were fed *ad lib.* and by force feeding and killed 12 hrs. after feeding. Samples of rectal contents were taken for analysis. SCERBINA and SORVACEV (1969) and PLAKAS

and KATAYAMA (1981) studied the amino acid availability in different parts of the alimentary tract of common carp (*Cyprius carpio*).

Information on the amino acid availability is of prime importance in understanding the nutritive utilization of food protein. As far as we know no such information has been published regarding the cod. The results presented here are based on a study on the use of chromium(III)-oxide and titanium(IV)-oxide as external indicator substances, and further the use of calcium as an internal indicator substance in nutrition experiments on captive and wild cod (LIED et al., 1982). The subsequent paper presents corresponding results on the nutritional availability of fats (LIED and LAMBERTSEN, 1982).

## METHODS

### *Experimental*

Cods weighing from 1.0 to 3.0 kg, obtained at different times from different localities off the western and northern coast of Norway were transferred to a sheltered 25 $m^3$ tank supplied with running sea water at 8°C and 35‰ salt. The photoperiod was regulated to 12 hrs. light and 12 hrs. dark, and the fish were acclimatised to the experimental conditions during 10 weeks. Whole capelin (*Mallotus villosus*) or sprat (*Sprattus sprattus*) were fed daily *ad lib.* during the acclimatisation. To avoid a change in the environment to which the fish had been acclimatised, the tank was divided into two compartments prior to the experimental period by a screen. Fish which fed well were selected for the experiment and isolated in one of the compartments. The fish were starved for 5 days to ensure an empty alimentary tract.

In Expt 1 70 fish ranging from 1.1 to 1.8 kg were force-fed moist pellets measuring 2 cm in diameter and 4 to 8 cm in length, consisting of 86% minced fish fillet from saithe (*Pollachius virens*), 7% fish oil and 7% dextrin, with 0,1% chromium(III)-oxide added to the mixture. Four cm of the moist pellet corresponded to 16 g feed, and each fish was fed 16 g per kg body weight. The gastric contents were collected at 4, 8 and 12 hrs., whereas the total gastrointestinal contents were sampled at 24, 36, 48 and 72 hrs. after feeding. Each sample comprised 10 randomly selected fish.

In Expt 2 8 cod averaging 1.5 kg were given whole sprat sorted out to be 16 cm long weighing on an average 15 g. Titanium(IV)-oxide gave a more stable suspension in water than chromium(III)-oxide, and was found more convenient in this experiment. Immediately before feeding 0.1 ml of a suspension of titanium(IV)-oxide was injected into the stomach of each feed fish giving a wet-weight concentration of the indicator substance of 0.5%. The cod were fed *ad lib.* twice daily for 9 days.

Finally 15 cods caught at three trawling stations on the Skolpen Bank (Barents Sea) were sampled aboard the R/V «G. O. Sars». All fish had fed on capelin as their sole food source. The water temperature was 3–4°C. Immediately upon the catch, the fish were gutted and the alimentary tract ligated into segments, removed from the fish and frozen. The frozen samples were brought to the laboratory, thawed and analysed as described below.

The gastrointestinal tract was divided into six segments by ligations at the oesophagus, immediately before the pyloric ceca, at the first cranial, the first caudal, the second cranial bend of the intestine, at the ileorectal valve, and at the anus. Starting with the stomach, the segments were numbered from one to six, representing 12, 6, 32, 30, 12 and 8% of the total length, respectively. Pooled samples were prepared and freeze dried as described by LIED et al. (1982). Portions were digested for the determination of nitrogen, chromium and titanium by the micro-Kjeldahl technique in a thermostat-regulated heating block (TECATOR 40). Nitrogen was determined in the digest by a salicylate-nitroprusside-isocyanurate reaction as described by CROOKE and SIMPSON (1971). Titanium(IV)-oxide was determined in the digest according to NJAA (1961).

Chromium was determined by atomic absorption spectrophotometry as described by LIED et al. (1982). Calcium was determined after nitric acid/perchloric acid digestion by AAS according to JULSHAMN and BRAEKKAN (1975) Samples for amino acid determination corresponding to 17 mg protein were hydrolysed with 60 ml 6 N HCl for 10 hrs. at 120°C. An aliquot was dried and diluted with 0.1 N HCl. The amino acids were determined on a TECHNICON NC-2P amino acid analyser system with a CHROMO-BEADS resin (NJAA and UTNE 1982).

The apparent nutrient recovery (ANR) relative to the indicator concentration either for nitrogen or amino acids was taken as a measure for apparent nutrient availability (ANA). The relationship between ANR and ANA is given by:

$$ANA = 100 - ANR$$

Percentage ANA was calculated by the formula:

$$ANA = 100 - 100\ (Sq/Iq)$$

where Iq and Sq are the ratios between nutrient concentration and indicator concentration in the feed and the gastrointestinal segment in question, respectively. In wild fish the calculation of availability was related to the gastric content of nutrients and calcium (LIED et al., 1982). The ratio Iq was replaced by Gq, which is the ratio nutrient concentration over calcium concentration in the stomach.

## RESULTS AND DISCUSSION

Morphological studies of the digestive tract of cod (BISHOP and ODENSE, 1966) and biochemical studies in samples from the pyloric ceca in cod (OVERNELL, 1973), trout (CROSTON, 1965; ASH, 1980), redfish (STERN and LOCKHART, 1953) chinook salmon (CROSTON, 1960) and mackerel (OOSHIRO, 1971) suggest that the pyloric ceca region of the intestine is a major area of enzyme secretion and absorption of digested proteins and other nutrients. Analyses of chyme and calculation of availability values in the indirect indicator method in different gastrointestinal sections further indicate the sites of absorption of nutrients. Using this method SCERBINA and SORVACEV (1969) found that 57% of arginine and 45% of phenylalanine were absorbed in the anterior 13% of the alimentary tract of carp. Similarly PLAKAS and KATAYAMA (1981) found in experiments with carp that 75% of the total amino acids in a casein diet was absorbed in the anterior intestine, including the oesophagus and functional stomach down to the first intestinal loop. DABROWSKI and DABROWSKA (1981) found that the major part of protein (N × 6.25) and amino acid absorption took place in the anterior and middle part of the intestine in the trout.

The experimental background for the results discussed here was given by LIED et al. (1982) where it was concluded that based on the methods given, the use of chromium(III)-oxide and titanium(IV)-oxide as external indicators and calcium as an internal indicator, were reliable in nutritional availability studies. The passage of chyme through the pyloric ceca region and into the anterior ileum reduced the concentration of nitrogen considerably and changed the composition of the N-fraction compared to that of the feed and gastric content (Table 1). In cod fed an easily digestible protein as minced saithe fillet (Expt 1) the fraction of nitrogen derived from amino acids (AA-fraction) decreased from 5.67% in the feed through 3.35% in the stomach and 1.88% in the pylorus ceca region to an anterior ileal concentration of 0.83%. Relative to chromium(III)-oxide, only 5.3% of the AA-fraction was recovered in segment 3 (Table 2), showing that 94.7% of the amino acid absorption took place in the gastric, pyloric and anterior ileal region of the digestive tract and mainly in the pyloric ceca. The absorbtion was negligible as the chyme passed through the succeeding segments of the ileum into the rectum. In cod ingesting whole fish the absorption of amino acids were completed in the posterior part of the ileum. The AA-fraction in dry matter in samples from Expt 2 was reduced from 5.61% in the feed to 1.61% in segment 5, leaving 10.2% of the amino acids unabsorbed relative to titanium(IV)-oxide (Tables 1 and 2). In samples from wild fish the AA-fraction was reduced from 5.86% in the stomach to 2.10% in segment 5, showing an apparant recovery of amino acids of 4.8% relative to the gastric levels and using calcium as the reference substance. Here again the main part of amino acid absorption took place in the pyloric ceca region.

Table 1. Amino acid N, taurine and total N in the feed and gastrointestinal contents of cod, expressed as percentage nitrogen of dry matter. Expt 1: diet of saithe, Expt 2: diet of whole sprat and wild cod: natural diet of capelin.

| | Feed | Segment[1] | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| *Expt 1* | | | | | | |
| Taurine | 0.05 | 0.01 | 0.85 | 0.24 | 0.12 | 0.09 |
| Amino acid N | 5.67 | 3.35 | 1.88 | 0.83 | 0.81 | 0.93 |
| Total N | 7.69 | 4.92 | 4.01 | 2.22 | 2.11 | 2.19 |
| *Expt 2* | | | | | | |
| Taurine | 0.05 | 0.03 | 0.56 | 0.32 | 0.26 | 0.13 |
| Amino acid N | 5.61 | 5.93 | 2.29 | 2.15 | 1.68 | 1.61 |
| Total N | 8.08 | 8.03 | 6.62 | 5.60 | 5.14 | 4.91 |
| *Wild cod* | | | | | | |
| Taurine | – | 0.03 | – | 0.32 | 0.29 | 0.12 |
| Amino acid N | – | 5.86 | – | 2.32 | 2.43 | 2.10 |
| Total N | – | 8.08 | – | 5.15 | 4.85 | 4.35 |

[1]) Description of segments: Table 2.

Table 2 Apparent recovery (ANR) of amino acid nitrogen from the stomach, the different segments of the intestine and the rectum from cod. Expt 1: Diet saithe fillet, Expt 2: diet whole sprat and wild fish: natural capelin diet. ANR defined as in text, page 55.

| Gastrointestinal segment | | Apparent recovery (%) | | |
|---|---|---|---|---|
| | | Expt 1 | Expt 2[e] | Wild fish[f] |
| Stomach | (S1) | 62.8[a] | 95.8 | – |
| Pyloric ceca | (S2) | 32.0±4.1[b] | 61.7 | – |
| Anterior ileum | (S3) | 5.3±0.9[b] | 30.9 | 19.0 |
| Middle ileum | (S4) | 4.0±0.4[c] | 11.4 | 9.1 |
| Posterior ileum | (S5) | 3.5±0.6[c] | 10.2 | 4.8 |
| Rectum | (S6) | 3.0[d] | 10.6 | 5.0 |

[a] Mean of 10 fish sampled at 36 hrs.
[b] Mean±SEM of 3 samples, each consisting of pooled digest from 10 fish.
[c] Mean±SEM of 4 samples, each consisting of pooled digest from 10 fish.
[d] Pooled sample from 10 fish collected at 72 hrs.
[e] Pooled sample from 8 fish collected at 36 hrs.
[f] Pooled samples from 15 fish.

The AA-fraction accounted for only a part of the total N in the feed and gastrointestinal contents. In Expt 1 the ratio amino acid N to total N (Kjeldahl) were 0.68, 0.47, 0.37, 0.38 and 0.42 in segments 1 to 5, respectively. The corresponding ratios in samples from Expt 2 were 0.74, 0.35, 0.38, 0.33 and 0.33. In wild fish the gastric ratio was 0.73 while the ratios of segments 3, 4 and 5 were 0.45, 0.50 and 0.48, respectively. A part of the non protein N-fraction was accounted for by taurine, which seemed to be secreted into the pyloric ileum. While negligible amounts of taurine were found in the gastric content of all fish samples, nitrogen derived from this substance made up 21% and 8.5% of total N in samples collected from the pyloric ceca region of cod fed saithe fillet and whole sprat, respectively (Table 1). Correspondingly, taurine made up 6.2% of the N-fraction from anterior ileum of wild cod. The level of taurine in the digest decreased as it passed on from the pyloric ceca region through the intestinal segments. The bile duct empties into the pyloric ileum (BISHOP and ODENSE, 1966). Either the sulphate esters of bile alcohols or conjugates of taurine constitute bile salt in fish (HASLEWOOD, 1967). The intestinal levels of taurine may therefore be explained as a secretion of bile salts into the pyloric ileum followed by reabsorption in the succeeding ileal segments. Further analyses of the composition of the non-protein N fraction could not be carried out due to the small amounts of chyme samples available. Some of the non-protein N may, however, orginate from the feed in the form of volatile bases such as ammonia, mono- di- and trimethylamine, and particularly as trimethylaminoxide. It is well known that marine fish muscle contains trimethylaminoxide (TMAO) (LOVE, 1970), and it has also been reported that the pyloric ceca of mackerel and sardine are rich in TMAO TAKADA and NISHIMOTO, 1958). Some of the non-protein N may further originate from nitrogen containing glycoproteins secreted into the intestine. If present in the hydrolysates, glycosamines as mannosamine and galactosamine will elute with cystine from the amino acid analysis column. In actual analyses of hydrolysates of intestinal chyme samples, a considerable amount of a ninhydrin-positive component was recorded as a single peak on the aminogram corresponding to cystine. This peak was absent in analyses of amino acids in hydrolysates of feeds and gastric contents. In Expt 1 this peak made up 8.2%, 17.1%, 16.6% and 19.1% of the total N fraction in chyme collected from the pyloric ceca region, the anterior ileum, the middle ileum and the posterior ileum, respectively. The corresponding values found in Expt 2 were 3.3%, 4.0%, 2.9% and 4.5%, all calculated as cystine.

Nitrogen derived from amino acids constitutes true protein-N. A general assumption when formulating fish feeds has been that protein (N × 6.25) digestibility values were indicative of available amino acids. Data from experiments on channel catfish, fed different commonly used vegetable feed ingredients, suggested a reasonable agreement between protein digestibility

Table 3. Apparent availability (ANA) of nitrogen in cod. The values are based on amino acid N and total N fraction in intestinal contents of segments 4 and 5. ANA defined as in text, p. 55.

| | Apparent availability | | |
|---|---|---|---|
| | Expt 1[a] | Expt 2[b] | Wild fish[c] |
| Amino acid N | 96.2±0.5 | 88.5 | 94.7 |
| Total N | 91.6±0.7 | 76.0 | 91.3 |

[a] Mean±SEM of 4 samplings (24, 36, 48 and 72 hrs.), each consisting of pooled samples from 10 fish.
[b] Mean of 8 fish.
[c] Mean of 15 fish.

values and available amino acid values (WILSON et al., 1981). However, in experiments with rainbow trout fed a fish meal diet, the apparent protein (N × 6.25) availability was measured to 80.5% in the rectum whereas the apparent availability values of the single amino acids except methionine and tryptophane varied between 82.0% and 90.9% (DABROWSKI and DABROWSKA, 1981).

In all samples from cod the apparent recovery of amino acid-N in the middle and posterior ileum was less than that of total N (Table 3). In Expt 1 the availability of the AA-fraction was 96.2 ± 0.5%, which was significantly different ($P < 0.05$) from the value of 91.6 ± 0.7% obtained for total N. The difference between availability values of total N and amino acid N was even more pronounced in Expt 2. calculated on total N and availability of 76.0% was obtained, whereas the availability value based on the AA-fraction was 88.5%, i.e. higher by 12.5%. The corresponding difference found in wild fish was 3.4%.

AUSTRENG (1978) reported that protein (N × 6.25) from a diet based on capelin meal fed to rainbow trout was partly absorbed in the stomach. Gastric absorption of single amino acids in rainbow trout has also been reported by DABROWSKI and DABROWSKA (1981), based on the indirect indicator method. For cod fed saithe fillet (Expt 1) the gastric availability values for the different amino acids obtained during the first 24 hrs. after feeding varied between 0 and 34% with an average availability of amino acid N of 4% to 9% (Table 4). The gastric absorption of single amino acids increased in samples collected at 36 and 48 hrs. and varied between 24 and 69%. The gastric absorption of amino acid-N was 37 and 41% and of total N 31 and 36%. These results show that estimates of gastric absorption of nutrients by the indirect indicator method are unreliable and strongly influenced by the sampling time relative to the feeding time. The estimated gastric absorption of nitrogenous nutrients

Table 4. Apparent availability of single amino acids, amino acid N and total N in the stomach of cod. Each value represents mean of 10 fish from Expt. 1

| Amino acid | Hrs. after feeding | | | | | |
|---|---|---|---|---|---|---|
| | 4 | 8 | 12 | 24 | 36 | 48 |
| Threonine | 3 | 1 | 25 | 11 | 40 | 38 |
| Valine | | | 9 | 9 | 39 | 31 |
| Methionine | | | | 2 | 37 | |
| Isoleucine | | | 9 | | 34 | 38 |
| Leucine | | | 16 | 2 | 35 | 38 |
| Phenylalanine | | | 13 | 7 | 35 | 29 |
| Lysine | | 2 | 9 | 2 | 40 | 44 |
| Arginine | | | | | 35 | 69 |
| Aspartic acid | 5 | 15 | 34 | 17 | 43 | 41 |
| Glutamic acid | | | 11 | 2 | 36 | 29 |
| Glycine | | | | | 25 | 24 |
| Serine | | | 12 | 8 | 40 | 33 |
| Alanine | | | 12 | 13 | 43 | 56 |
| Tyrosine | | | 6 | 2 | 27 | 27 |
| Histidine | | | 4 | | 37 | 44 |
| Amino acid N | | | 9 | 4 | 37 | 41 |
| Total N | | | 6 | | 31 | 36 |

in our experiments may be caused by the retention of indicator substance relative to solubilised proteins and amino acids as well as by a variable filling of the intestinal tract (EDIN, 1926). However, the possibility of gastric absorption of amino acids and peptides in fish cannot be rejected.

In cod fed saithe fillet (Expt 1) the mean availability of the different amino acids varied between 94.3% and 97.6% (Table 5). In cod fed whole sprat (Expt 2) the availabilities of amino acids were somewhat lower with values between 83.8% and 93.1%.In wild fish, in which the calculation of availability was related to the gastric levels of calcium and amino acids the apparent availabilities were in the range 91.0% to 96.0%. The highest availability value was obtained for lysine in the three experiments.

In conclusion one may state that calculations of protein digestibility based on the values of total N × 6.25 in feed and gastrointestinal contents will bias the estimates of protein and protein availabilities due to the presence of non-protein N. Non-protein nitrogen includes taurine from the bile excretion, and TMAO, other amino bases and glycosamines secreted into the intestine. Amino acid N should be used to estimate protein digestibility in fish fed fresh fish protein (fish and fish viscera). According to the values given in Table 5, one may suggest that an estimation of protein digestibility could also be determined as single amino acid recoveries.

Table 5. Mean apparent availability of amino acids in cod. Expt. 1: fed saithe fillet, Expt 2: fed whole sprat and wild cod:fed capelin. The values are based on segments 4 and 5 of the intestine.

| Amino acid | Expt 1[a] | Expt 2[b] | Wild fish[c] |
|---|---|---|---|
| Threonine | 94.6±0.7 | 84.3 | 94.0 |
| Valine | 95.5±0.3 | 87.1 | 91.0 |
| Methionine | 94.3±0.5 | 89.3 | 94.1 |
| Isoleucine | 96.0±0.5 | 87.2 | 92.5 |
| Leucine | 96.7±0.3 | 88.3 | 94.1 |
| Phenylalanine | 97.5±0.4 | 90.4 | 94.5 |
| Lysine | 97.6±0.3 | 89.8 | 96.0 |
| Arginine | 96.9±0.3 | 89.8 | 95.0 |
| Aspartic acid | 95.6±0.5 | 85.6 | 95.3 |
| Glutamic acid | 95.9±0.3 | 93.1 | 95.7 |
| Glycine | 94.7±0.5 | 81.6 | 93.5 |
| Serine | 94.4±0.5 | 83.8 | 93.6 |
| Alanine | 96.5±0.3 | 91.4 | 95.1 |
| Tyrosine | 94.7±0.5 | 88.7 | 93.2 |
| Histidine | 95.5±0.3 | 90.9 | 94.0 |

[a] Mean±SEM of 4 samplings (24, 36, 48 and 72 hrs.), each consisting of pooled samples from 10 fish.
[b] Mean of 8 fish.
[c] Mean of 15 fish.

It has been generally accepted that animal proteins fed to carnivorous fish are assimilated to an extent greater than 90% (PHILLIPS, 1969; COWEY and SARGENT, 1972; KAPOOR et al., 1975). The availability values from Expt. 1, Expt 2 and from wild fish (Table 3) correspond well with literature data. In fish fed saithe fillet 96.2 ± 0.5% of the AA-fraction was absorbed. Corresponding values from cod on a natural capelin diet and cod fed whole sprat were 94.7% and 88.5%, respectively.

ACKNOWLEDGEMENTS

We thank Mr. O. M. Smedstad for collecting samples from wild cod and Mr. R. Solbakken and Mr. G. Yven for technical assistance. This research was supported by the Norwegian Fisheries Research Council grant I 711.10.

## REFERENCES


ASH, R., 1980. Comp. Biochem. Physiol. *65B*, 173–176.

AUSTRENG, E., 1978. Aquaculture *13*, 265–272.

BISHOP, C. and ODENSE, P. H., 1966. J. Fish. Res. Bd. Canada *23*, 1607–1630.

COWEY, C. B. and SARGENT, J. R., 1972. Adv. Mar. Biol. *10*, 383–492.

CROOKE, W. M. and SIMPSON, W. E., 1971. J. Sci. Food Agric. *22*, 9–10.

CROSTON, C. B., 1960. Archs. Biochem. Biophys. *89*, 202–206.

CROSTON, C. B., 1965. Archs. Biochem. Biophys. *112*, 218–223.

DABROWSKI, K. and DABROWSKA, H., 1981. Comp. Biochem. Physiol. *69A*, 99–111.

EDIN, H., 1926. Meddelande N:r 165 från Centralanstalten på jordbruksområdet, Husdjursavdelingen N:r 50. Sthlm. 77 pp.

HASLEWOOD, G. A. D., 1967. J. Lip. Res. *8*, 535–550.

JULSHAMN, K. and BRAEKKAN, O. R., 1975. At. Absorp. Newsl. *14*, 49–52.

KAPOOR, B. G., SMIT, H. and VERIGHINA, I. A., 1975. Adv. Mar. Biol. *13*, 109–239.

LIED, E., JULSHAMN, K. and BRAEKKAN, O. R., 1982. Can.J. Fish. Aquat. Sci. *39*, 854–861.

LIED, E. and LAMBERTSEN, G., 1982. Fisk. Dir. Skr., Ser. Ernæring, *2*.

LOVE, R. M., 1970. «The Chemical biology of Fishes», Academic Press, London and New York.

NJAA, L. R., 1961. Acta Agric. Scand. *11*, 227–241.

NJAA, L. R. und UTNE, F., 1982. Fisk. Dir. Skr., Ser. Ernæring, *2*, 25–33.

OOSHIRO, Z., 1971. Bull. Jap. Soc. Scient. Fish. *37*, 145–148.

OVERNELL, J., 1973. Comp. Biochem. Physiol. 46B, 519–531.

PHILLIPS, A. R. jr., 1969. In «Fish Physiology» (Hoar, W. S. and Randall, D. J., eds.), Vol *1*, 351–432. Academic Press, New York.

PLAKAS, S. M. and KATAYAMA, T., 1981. Aquaculture, *24*, 309–314.

SCERBINA, M. A. and SORVACEV, K. F., 1969. J. Ichthyol., *16*, 315–322.

STERN, J. A. and LOCKHART, E. E., 1953.J. Fish. Res. Bd. Canada *10*, 590–598.

TAKADA, K. and NISHIMOTO, J., 1958. Bull. Jap. Soc. Scient. Fish. *24*, 623–635.

WILSON, R. P., ROBINSON, E. H. and POE, W. E., 1981. J. Nutr. *111*, 923–929.