

ORIGINAL ARTICLE

Amylase and Trypsin Activity in an Economically Important Fish *Labeo Calbasu* of Tripura, India

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Abstract:

Labeo calbasu is considered as one of the preferred fish in the market of different corners of India. Certain biochemistry profile of this fish species at its different life cycle stages has been analysed. The digestive enzyme activity shows the effective role of amylase (936.00-1014.00 in activity unit per 100 g fish body weight) and trypsin (44.00-62.00 in activity unit per 100 g fish body weight) in *Labeo calbasu*. With the examination of faecal matter from its stomach, fore-gut, hind-gut and anus area of the fish sample it depicts variation of apparent digestibility coefficient in its pre-adult as well as adult stage. Pre-adult stage exhibits excretion of 20% protein in the faecal matter while adult stage showing excretion of 40% protein in the faeces. Metabolic faecal protein during the adult stage of the fish amounting 3.6 g/100g of the food consumed. So, the true digestibility coefficient of the protein has been amounted to about 100% and the amount of food ingested by the adult fish has been about 100g. Therefore, amongst 100% protein availability in the consumed food the metabolic protein has been 3.6 g, digestible protein is 96.4 g while the apparent digestible coefficient is about 96.4%. When the adult fish sample is being provided rice powder as feed at different quantities the digestibility coefficient % also found to be varied. When feeding level has been 2 g/fish/day the nutrient composition has been 86.2% protein, 87.66% lipids, 86.78 % fibres and 88.12% nitrogen free extracts as well. Similarly, the digestibility coefficient % varies in relation to the variations of nutrient composition.

Key words: *Labeo calbasu*, life cycle stage, digestibility coefficient, amylase, trypsin

Introduction

Knowledge on fish biochemistry is important to study fish growth in commercial view point. Amongst various fish species carps are considered to be significant since they are capable to meet the demand of human being as highly digestible proteinaceous food. Biochemistry profile of different carps are although known. However, knowledge on *Labeo calbasu* is poor in literature (Banik 2021). Further, digestive enzyme activity of many fish species is poorly understood (Kitamikado and Tachino 1960). It varies with fish age, physiological state and seasonal climatic character. For proper growth and development of fish fauna knowledge of digestive enzyme is very

important (Kitamikado and Tachino 1960). Simultaneously, digestion and absorption of a particular kind of food also directly promotes growth rate of a fish at different life cycle stages. A survey into the literature explicitly reveals that information on the digestibility coefficient of fish fauna is poor (Abel 1983, Abel *et al.* 1985, 1986, Agustsson and Strum 1981, Ahokas and Sorg 1977, Amano and Yamada 1964a, b, Anderson 1980, 1986, Anderson and Casey 1984, Aonuma *et al.* 1969, Avena and Bowen 1969, Baker and Chaykin 1962, Baker *et al.* 1963, Ballantyne *et al.* 1986, Baslow 1965, 1966, 1967, Baslow and Lenney 1967, Bate-Smith 1938, Bauermeister *et*

al. 1983, Beatty 1938, Bell 1987, Benoit and Norris 1945, Bergeret and Chatagner 1956, Bocharnikova and Petushkova 1967, Bogerd *et al.* 2001, Boime. and Ben-Menahem 1999, Boldyrev and Lebedev 1967, Bousfield *et al.* 1994, Boyd *et al.* 1977, Brown 1981, Campbell *et al.* 1983, Cowey *et al.* 1962, 1981, Cowey and Parry 1963, Casey and Anderson 1982, Casey and Anderson 1985. Castell *et al.* 1971, 1973, Castellini and Somero 1981, Cholette and Gagnon 1973, Daikoku, T. and Sakaguchi, M. 1983, Daikoku *et al.* 1987, Forster and Goldstein 1976, Forster *et al.* 1978, Fowler *et al.* 1979, Gen *et al.* 2000, Gill and Paulson 1982, Gomez *et al.* 1999, Gothilf *et al.* 1997).

The present observation communicates analysis of amylase, trypsin and digestibility coefficients of protein during pre-adult and adult stages of *Labeo calbasu*.

Materials and Methods

For experimentation with the studied fauna live fish samples during pre-adult fingerling and adult stages are being considered. In order to analyse

different biochemical profile of *Labeo calbasu* the literature of Hephher (1988) has been followed here.

Results and Discussion

The digestive enzyme activity in *Labeo calbasu* depicts effective role of amylase (976.00-1014.0 in activity unit per 100 g of fish body weight) and trypsin (54.00-62.00 in activity unit per 100 g of fish body weight) during its adult stage (figs 1-2). However, during pre-adult stage of the fish sample both the values of amylase (936.00-966.00 in activity unit per 100 g of fish body weight) and trypsin (44.00-52.00 in activity unit per 100 g of fish body weight) are found to be relatively low (figs. 3-4). Similar observations are also being noticed in the common carp *Cyprinus carpio* by some researchers (Shimeno and Ikeda 1967 and Shimeno *et al.* 1979). However, Kitamikado and Tachino (1960a, b, c) pointed out that the amylolytic activity in trout has been relatively greater during its pre-adult and adult stages.

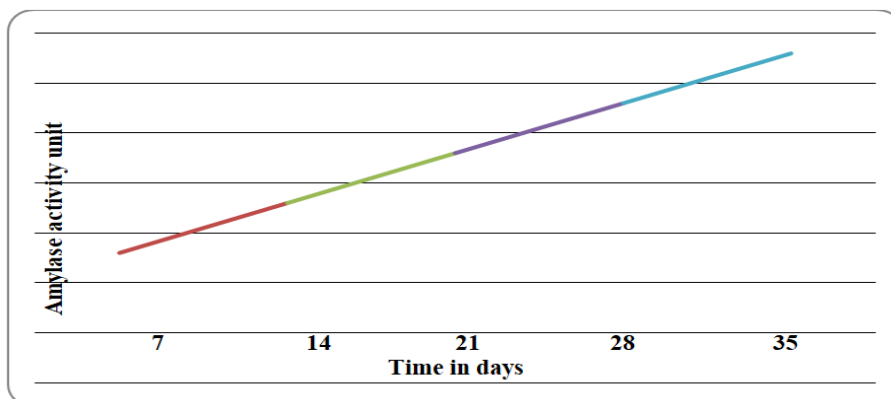


Fig. 1: Amylase activity in *Labeo calbasu* during adult stage

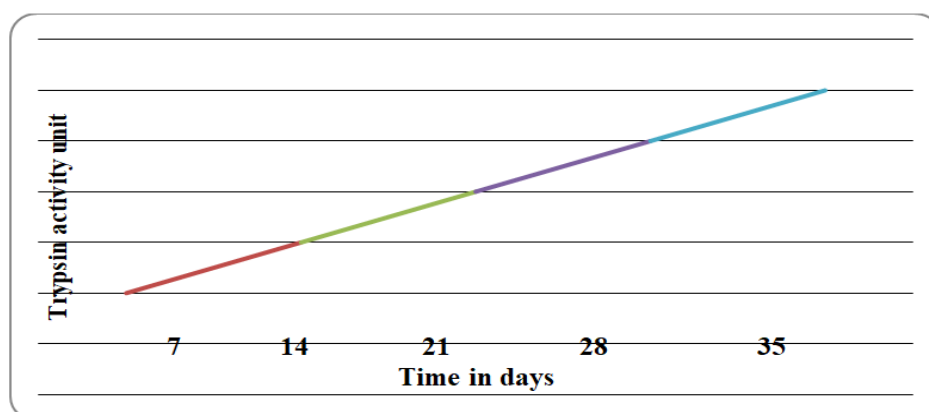


Fig. 2: Trypsin activity in *Labeo calbasu* during adult stage

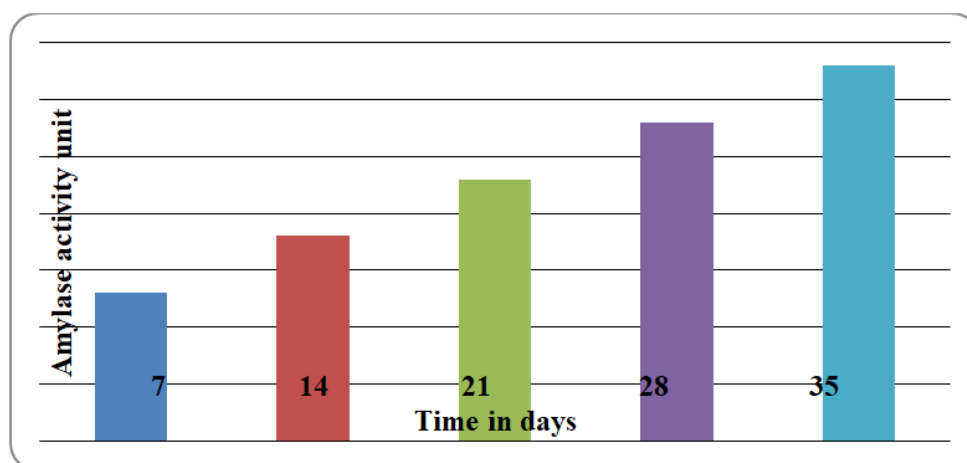


Fig.3: Amylase activity in *Labeo calbasu* during pre-adult stage

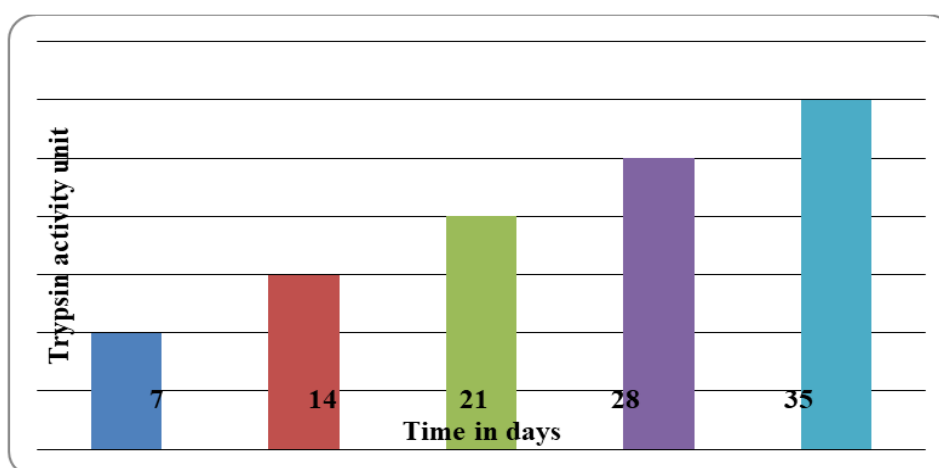


Fig. 4: Trypsin activity in *Labeo rohita* during pre-adult stage

During studies of apparent digestibility coefficients of protein by *Labeo calbasu* at two protein levels and for faeces collected at different areas of the digestive tract the results depict that with the assessment of faecal matter from its stomach, fore-gut, hind-gut and anus area the adult fish species exhibits variation of apparent digestibility coefficient during its fingerling and adult stage as well. Fingerling stage of the fish depicts excretion of 20% protein in the faecal

matter while the adult stage showing excretion of 40% protein in the faeces (tables 1, 1A-2 and 3-4). The values have been relatively greater in the studies of Austreng (1978) where faeces have been sampled from the posterior areas of anus of the fish sample. Similar to that of Austreng (1978) several observations have also been reported from the experiments of Austreng and Refstie (1979), Smith and Lovell (1971, 1973) which are in line with the present results.

Table 1: Analysis of apparent digestibility coefficients of protein during adult stage of *Labeo calbasu* at two protein levels through the faeces (%) sampled at different areas of the digestive tract

| Three Protein level | Faeces collected from different areas of digestive system | | | |
|---------------------|---|----------|----------|-------|
| | Stomach | Fore gut | Hind gut | Anus |
| 20% | 61.78 | 64.88 | 74.86 | 81.12 |
| 40% | 61.22 | 74.00 | 86.24 | 91.62 |
| 60% | 60.00 | 78.20 | 88.66 | 94.14 |

Table 1A: Analysis of apparent digestibility coefficients of protein during pre-adult stage of *Labeo calbasu* at two protein levels through the faeces (%) sampled at different areas of the digestive tract

| Three Protein level | Faeces collected from different areas of digestive system | | | |
|---------------------|---|----------|----------|-------|
| | Stomach | Fore gut | Hind gut | Anus |
| 20% | 52.00 | 46.00 | 64.00 | 68.00 |
| 40% | 50.00 | 62.00 | 73.00 | 78.00 |
| 60% | 48.00 | 66.00 | 74.00 | 82.00 |

Experiments on digestibility coefficient of the protein exhibits that metabolic faecal protein during the pre-adult stage of the fish amounting 3.6 g/100g of the food consumed. Hence, the true digestibility coefficient of the protein has been about 100% and the amount of food ingested by the adult fish is about 100g. Thus, amongst 100% protein availability in the consumed food content the metabolic protein is about 3.6 g, digestible protein is 96.4 g while the apparent digestible coefficient has been 96.4% (table 2). Similarly,

the trend of apparent digestible coefficient has been relatively lower when the percentage of protein food is also lower. Nose (1964) also noticed that the apparent digestible coefficient in some Japanese fish has been greater when the percentage of protein food is also greater. Nose (1964, 1971) further pin pointed that the apparent digestible coefficient will be lower when the fish is provided with lower percentage of protein food. Similar observations have also been noticed by Banik (2020, 2022).

Table 2: Analysis of apparent digestibility coefficient of the protein at pre-adult stage of fish

| Protein food (%) | Metabolic protein (g) | Digestible protein (g) | Apparent digestible coefficient (%) |
|------------------|-----------------------|------------------------|-------------------------------------|
| 100 | 3.6 | 96.4 | $96.4 \times 100/100 = 96.4$ |
| 50 | 3.6 | 49.0 | $49.0 \times 100/50 = 98.0$ |
| 20 | 3.6 | 16.0 | $16.0 \times 100/20 = 80.0$ |
| 10 | 3.6 | 07.4 | $07.4 \times 100/10 = 74.0$ |

Table 3: Analysis of digestibility coefficient (%) of rice powder provided to *Labeo calbasu* during its adult stage.

| Feeding level (g/fish/day) | Protein | Lipid | Fibres | Nitrogen free extract |
|----------------------------|---------|-------|--------|-----------------------|
| 2 | 86.2 | 87.66 | 86.78 | 88.12 |
| 4 | 80.14 | 84.86 | 88.11 | 78.89 |
| 6 | 88.24 | 93.46 | 94.21 | 93.43 |

Table 4: Digestibility coefficient (%) of rice powder provided to *Labeo calbasu* at different pre-adult stage of fish

| Feeding level (g/fish/day) | Protein | Lipid | Fibres | Nitrogen free extract |
|----------------------------|---------|-------|--------|-----------------------|
| 2 | 76.26 | 82.43 | 84.47 | 72.68 |
| 4 | 80.00 | 83.66 | 86.88 | 78.82 |
| 6 | 86.22 | 90.88 | 92.89 | 94.12 |

Banik (2021) noticed that the metabolic faecal protein is found to be proportional to the amount of food ingested by the fish. So, the apparent digestibility coefficient of the protein will usually

increase with increase in the amount of protein in the food. When the adult fish sample is provided rice powder as feed at different quantities the digestibility coefficient % has also been varied

accordingly. Similarly, the digestibility coefficient % varies in relation to the variations of feeding level from 4 to 6 g/fish sample/day where nutrient composition has also been varied (table 4). Several workers (Bondi and Spandorf 1953, 1954, Bondi *et al.* 1957) also observed similar results in their analysis and pointed out that the digestibility coefficient correlates to the quantitative variations of food provided to the fish sample.

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References

- Abel, H. 1983. Distribution of free L-histidine and related dipeptides in the muscle of freshwater fishes. *Comp. Biochem. Physiol.* 76B, 35-40. Fish non-protein nitrogenous compounds 223.
- Abel, H., Dobson, P. G., Hoeger, U. and Parkhouse, W. S. 1985. The role of histidine and histidine-related compounds to intracellular buffering in fish skeletal muscle. *Am. J. Physiol.* 249, R449-R454.
- Abel, H., Brill, R. W. and Hochachka, P. W. 1986. Metabolism of L-histidine, carnosine and anserine in skipjack tuna. *Physiol. Zool.* 59, 385-397.
- Agustsson, I. and Strum, A. R. 1981. Biosynthesis and turnover of trimethylamine oxide in the teleost cod, *Gadus morhua*. *J. biol. Chem.* 256, 8045-8049.
- Ahokas, R. A. and Sorg, G. 1977. The effect of salinity and temperature on intracellular osmoregulation and muscle free amino acids in *Fundulus diaphanus*. *Comp. Biochem. Physiol.* 56A, 101-105.
- Amano, K. and Yamada, K. 1964. A biological formation of formaldehyde in the muscle tissue of gadoid fish. *Bull. Jap. Soc. scient. Fish.* 30, 430-435.
- Amano, K. and Yamada, K. 1964b. Formaldehyde formation from trimethylamine oxide by the action of pyloric caeca of cod. *Bull. Jap. Soc. scient. Fish.* 30, 639-4345.
- Anderson, P. M. 1980. Glutamine- and N-acetylglutamate dependent carbamoyl phosphate synthetase in elasmobranchs. *Science* 208, 291-293.
- Anderson, P. M. and Casey, C. A. 1984. Glutamine dependent synthesis of citrulline by isolated hepatic mitochondria from *Squalus acanthias*. *J. biol. Chem.* 259, 456-462.
- Anderson, P. M. 1986. Effects of dietary trimethylamine oxide and osmolality on respiration and citrulline synthesis by isolated hepatic mitochondria from *Squalus acanthias*. *Comp. Biochem. Physiol.* 3513, 783-788.
- Aonuma, S., Hama, T., Tamaki, N. and Okumura H. (1969) Orotate as a fl-alanine donor for anserine and carnosine biosynthesis, and effects of actinomycin D and azauracil on their pathway. *J. Biochem., Tokyo* 66, 123-132.
- Austreng, E. 1978. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tracts. *Aquaculture* 13: 265-272.
- Austreng, E. and Refstie, T. 1979. Effect of varying dietary protein level in different families of Rainbow trout. *Aquaculture* 18: 145-156.
- Avena, R. M. and Bowen, W. J. 1969. Effects of carnosine and anserine on muscle adenosine triphosphatase. *J. biol. Chem.* 224, 1600-1604.
- Banik, R. 2020. Biochemical characters of some herbivore, omnivore and carnivorous fish species of Tripura. India. Unpublished data, MSc dissertation, Department of Molecular Biology & Bioinformatics, Tripura University, 47 pages,
- Banik, R. 2021. Some aspects of biochemistry of a near threatened fish *Chitala chitala* of Tripura. India. Unpublished data of MSc dissertation, Department of Molecular Biology & Bioinformatics, Tripura University,
- Banik, R. 2022. Biochemistry of an endangered fish *Ompok bimaculatus* of Tripura. India. Unpublished data of MSc dissertation, Department of Molecular Biology & Bioinformatics, Tripura University,
- Baker, J. R. and Chaykin, S. 1962. The biosynthesis of trimethylamine N-oxide. *J.*

- biol. Chem. 237, 1308-1313.
19. Baker, J. R., Struempfer, A. and Chaykin, S. 1963. A comparative study of trimethylamine N-oxide biosynthesis. *Biochim. biophys. Acta* 71, 58-84.
 20. Ballantyne, J. S., Moyes, C. and Moon, T. W. 1986. Osmolarity affects oxidation of sarcosine by isolated hepatocytes and mitochondria from a euryhaline elasmobranch. *J. exp. Zool.* 238, 267-271.
 21. Baslow, M. H. 1965. Neurosine, its identification with N-acetylhistidine and distribution in aquatic vertebrates. *Zoologica* 50, 63-66.
 22. Baslow, M. H. 1966. N-acetyl-L-histidine synthetase activity from the brain of the killifish, *Fundulus heteroclitus*. *Brain Res.* 3, 210-213.
 23. Baslow, M. H. 1967. N-acetyl-L-histidine metabolism in the fish eye: evidence for ocular fluid-lens L-histidine recycling. *Exptl. Eye Res.* 6, 336-342.
 24. Baslow, M. H. and Lenney, J. F. 1967. N-acetyl-L-histidine aminohydrolase activity from the brain of skipjack tuna, *Katsuwonus pelamis*. *Can. J. Biochem.* 45, 337-340.
 25. Bate-Smith, E. C. 1938. The buffering of muscle in rigor: protein, phosphate and carnosine. *J. Physiol., Lond.* 92, 336-343.
 26. Bauermeister, A., Lewendon, A., Ramage, P. I. N. and Nimmo I. A. 1983. Distribution and some properties of the glutathione S-transferase and 7-glutamyl transpeptidase activities of rainbow trout. *Comp. Biochem. Physiol.* 74C, 89-93.
 27. Beatty, S. A. 1938. Studies of fish spoilage II. The origin of trimethylamine produced during the spoilage of cod muscle press juice. *J. Fish. Res. Bd Can.* 4, 83-88.
 28. Bell, J. G., Buddington, R. K., Walton, M. J. and Cowey, C. B. 1987. Studies on the putative role of γ -glutamyl transpeptidase in intestinal transport of amino acids in Atlantic salmon. *J. comp. Physiol.* 157, 161-169.
 29. Benoit, G. J. and Norris, E. R. 1945. Studies on trimethylamine oxide II. The origin of TMAO in young salmon. *J. biol. Chem.* 158, 438-442.
 30. Bergeret B. and Chatagner F. 1956. Etude des d6carboxylations de l'acidecysteinesulphinique et de l'acideglutamique dans le foie et le cerveau du rat, et de la rate ovarioectomisee. Absence de d6carboxylations chez certains animaux marins. In *Colloque sur la Biochimie du Soufre*, pp. 118-122.
 31. Bocharnikova, I. M. and Petushkova, E. V. 1967. Effect of imidazole compounds on the ATPase activity of myosin during heat denaturation and in the presence of certain reagents. *Biochemistry, USSR* 32, 99-103
 32. Bogerd, J., Blomenrohr, M., Andersson, E., van der Putten, H.H.A.G.M., Tensen, C.P., Vischer, H.F., Granneman, J.C.M., Janssen-Dommerhold, C., Goos, H.J.Th. and Schulz, R.W. 2001 Discrepancy between molecular structure and ligand selectivity of testicular follicle-stimulating hormone receptor of the African catfish (*Clarius gariepinus*). *Biol. Reprod.* 64: 1633-164
 33. Boime, I. and Ben-Menahem, D. 1999. Glycoprotein hormone structure-function and analog design. *Recent Prog. Horm. Res.* 54: 217-288.
 34. Boldyrev, A. A. and Lebedev, A. V. 1967. Influence of imidazole and carnosine on the working capacity of a tetanic muscle during direct stimulation, *Biochemistry, USSR* 32, 495-500
 35. Bondi, A. and Spandorf, A. 1953. The activity of digestive enzymes of the carp. In *Hebrew: Bamidgeh* 5 (7-8): 116-130.
 36. Bondi, A. and Spandorf, A. 1954. The action of the digestive enzymes of the carp. *Br. J. Nutr.* 8: 240-246.
 37. Bondi, A., Spandorf, A. and Calmi, R. 1957. The nutritive value of various feeds for carp. *Bamidgeh* 9 (1): 13-18.
 38. Bousfield, G.R., Perry, W.M. and Ward, D.N. 1994. Gonadotropins: chemistry and biosynthesis. In: *The Physiology of Reproduction*, pp. 1749-1792
 39. Boyd, T. A., Cha, C., Forster, R. P. and Goldstein, L. 1977. Free amino acids in tissues of the skate *Raja erinacea* and the stingray *Dasyatis sabina*: effects of environmental dilution. *J. exp. Zool.* 199, 435-442
 40. Brown, C. E. 1981. Interactions among carnosine, anserine, ophidine and copper in biochemical adaptation. *3. theor. Biol.* 88, 245-256
 41. Campbell, J. W., Aster, P. L. and Vorhaben, J. E. 1983. Mitochondrial ammoniogenesis in the channel catfish, *Ictalus punctatus*. *Am. J.*

- Physiol. 244, R709-R717
42. Cowey, C. B., Cooke, D. J., Matty, A. J. and Adron, J. W. 1981. Effect of quantity and quality of dietary protein on certain enzyme activities in rainbow trout. *J. Nutr.* 111, 336-345.
 43. Cowey, C. B., Daisley, K. W. and Parry, G. 1962. Study of amino acids, free or as components of proteins, and of some B vitamins in the tissues of the Atlantic salmon, *Salmo salar*, during spawning migration. *Comp. Biochem. Physiol.* 7, 28-38.
 44. Cowey, C. B. and Parry, G. 1963. The non-protein nitrogenous constituents of the muscle of parr and smolt stages of the Atlantic salmon (*Salmo salar*). *Comp. Biochem. Physiol.* 8, 47-51
 45. Casey, C. A. and Anderson, P. M. 1982. Subcellular localization of glutamine synthetase and urea cycle enzymes in liver of spiny dogfish, *Squalus acanthias*. *J. Biol. Chem.* 257, 3449-8453
 46. Casey, C. A. and Anderson, P. M. 1985. Submitochondrial localization of arginase and other enzymes associated with urea synthesis and nitrogen metabolism in liver of *Squalus acanthias*. *Comp. Biochem. Physiol.* 32B, 307-315
 47. Castell, C. H., Smith, B. and Neal W. 1971. Production of dimethylamine in muscle of several species of Gadoid fish during frozen storage, especially in relation to presence of dark muscle. *J. Fish. Res. Bd Can.* 28, 1-5
 48. Castell, C. H., Neal, W. E. and Dale, J. 1973. Comparison of changes in trimethylamine, dimethylamine and extractable protein in iced and frozen fish fillets. *J. Fish. Res. Bd Can.* 30, 1246-1248
 49. Castellini, M. A. and Somero, G. N. 1981. Buffering capacity of vertebrate muscle: correlations with potentials for anaerobic function. *J. comp. Physiol.* 143, 191-198
 50. Cholette, C. and Gagnon, A. 1973. Isosmotic adaptation in *Myxine glutinosa* L. II. Variations of the free amino acids, trimethylamine oxide and potassium of the blood and muscle cells. *Comp. Biochem. Physiol.* 45A, 1008-1021
 51. Daikoku, T. and Sakaguchi, M. 1983. Effects of dietary trimethylamine on free amino acid and nonprotein nitrogen levels in muscle of the guppy, *Poecilia reticulata*, in relation to sea-water adaptation. *Comp. Biochem. Physiol.* 75A, 343-346
 52. Daikoku, T., Arai, S., Murata, M. and Sakaguchi, M. 1987. Effects of dietary trimethylamine on the concentration of trimethylamine oxide in various tissues of the chum salmon, *Oncorhynchus keta*, in freshwater and sea-water environments. *Comp. Biochem. Physiol.* 77A, 101-105
 53. Forster, R. P., Hannafin, J. A. and Goldstein, U. 1978. Osmoregulatory role of amino acids in brain of the elasmobranch, *Raja erinacea*. *Comp. Biochem. Physiol.* 60A, 25-30.
 54. Fowler, B. J., Allen, R. L. and Phipps, D. A. 1979. Isolation of I-histidine ammonia lyase from rainbow trout. *Biochem. Soc. Trans.* 7, 1081-1083.
 55. French, J., Mommsen, T. P. and Hochachka, P. W. 1981. Amino acid utilization in isolated hepatocytes from rainbow trout. *Eur. J. Biochem.* 113
 56. Forster, R. P. and Goldstein, U. 1976. Intracellular osmoregulatory role of amino acids and urea in marine elasmobranchs. *Am. J. Physiol.* 231, 925-931.
 57. Gen, K., Okuzawa, K., Senthilkumaran, B., Tanaka, H., Moriyama, S. and Kagawa, H. 2000. Unique expression of gonadotropin I and II subunits in male and female red seabream (*Pagrus major*) during sexual maturation. *Biol. Reprod.* 63: 308-319.
 58. Gill, T. A. and Paulson, A. T. 1982. Localization, characterization and partial purification of TMAO-ase. *Comp. Biochem. Physiol.* 71B, 49-56.
 59. Gomez, J.M., Weil, C., Ollitrault, M., Le Bail, P.-Y., Breton, B. and Le Gac, F. 1999. Growth hormone (GH) and gonadotropin subunit gene expression and pituitary and plasma changes during spermatogenesis and oogenesis in rainbow trout (*Oncorhynchus mykiss*). *Gen. Comp. Endocrinol.* 113: 413-423
 60. Gothilf, Y., Meiri, I., Elizur, A. and Zohar, Y. 1997. Preovulatory changes in the levels of three gonadotropin-releasing hormone mRNAs, gonadotropin β subunit mRNAs, plasma gonadotropin, and steroids in the female gilthead seabream, *Sparus aurata*. *Biol. Reprod.* 57: 1145-1154

61. Hopher, B. 1988. Nutrition of Pond Fishes. Oxford publication, 388 pages,
62. Kitamikado, M. and Tachino, S. 1960a. Studies on the digestive enzymes of rainbow trout. I. Carbohydrates. Bulletin of Japanese Society of Science Fish 26: 679-684.
63. Kitamikado, M. and Tachino, S. 1960b. Studies on the digestive enzymes of rainbow trout. II. Proteases. Bulletin of Japanese Society of Science Fish 26: 685-690.
64. Kitamikado, M. and Tachino, S. 1960c. Studies on the digestive enzymes of rainbow trout. III. Esterases. Bulletin of Japanese Society of Science Fish 26: 691-694.
65. Nose, T. 1964. Protein digestibility of several test diets in cray and prawn fish. Bulletin of Freshwater Fish Res. Lab., Tokyo 14:23-28
66. Nose, T. 1967. Recent advances in the study of fish digestion in Japan. FAO/EIFAC Technical paper 3:83-94
67. Nose, T. 1971. Determination of nutritive value of food protein in fish. III. Nutritive value of casein, whitefish meal and soybean meal in rainbow trout fingerlings. Bulletin of Freshwater Fish Res. Lab., Tokyo 21: 85-98.
68. Shimeno, S. and Ikeda, S. 1967. Studies on glucose-6-phosphatase of aquatic animals. II. The enzyme activities in fish tissues. Bulletin of Japanese Society of Science Fish 33: 112-116.
69. Shimeno, S., Hosokawa, H. and Takeda, M. 1979. The importance of carbohydrate in the diet of carnivorous fish. In: J. E. Halver and K. Tiews (eds). Finfish nutrition and fishfeed technology, Vol. I pp. 127-143, Heenemann Verlagsgesell publ, Berlin
70. Smith, B. W. and Lovell, R. T. 1971. Digestibility of nutrients in semi-purified rations by channel cat fish in stainless steel troughs. Proc. Ann. Conference of Southeast Association on Game fish Commercialisation. 25: 452-459.
71. Smith, B. W. and Lovell, R. T. 1973. Determination of apparent protein digestibility in feeds for channel cat fish. Trans. Am. Fish Soc. 102: 831-835.