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Differential utilization of proteins in *Anabas testudineus*(Bloch) exposed to brief and prolonged fasting

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Abstract
In the present study total proteins were investigated and estimated in *Anabas testudineus* (Bloch) which was exposed to brief (15days) and prolonged fasting (60days).Method developed by Lowry *et.al* (1951) was adopted to estimate the total proteins. Six tissues were selected for the study. The results showed differential utilization of protein in selected tissues. (liver, kidney, brain, accessory respiratory organ, pectoral and lateral line muscle).There was an overall decline observed in the protein levels in most of tissues, with the exception being liver, brain and accessory respiratory organ which showed an increase in protein levels. This increase also was not found to be uniform. Liver and brain showed an elevation only during long term fasting and accessory respiratory organ showed an insignificant increase during both the fasting regimes. This significant depletion of total proteins in most of the tissues of *Anabas* during both the fasting regimes, suggests protein utilization to satisfy the energy demands and to combat the stressful condition and thus prolonging the longevity of the starving *Anabas.*

Keywords: Proteins, *Anabas*, starvation stress, gluconeogenesis, fasting, hormones

INTRODUCTION

It is interesting and fascinating to note that fishes have an extra ordinary ability to with stand prolonged periods of starvation .Many species of teleosts demonstrate the ability to withstand prolonged periods of starvation (Larson and Lewander, 1973; Loughna and GoldSpink; Machado *et.al*, 1988). Starvation is known to be tolerated by many species of fish both in and their natural environment during migrations reproduction (Hinch *et.al*, 2005, Miller *et.al*, 2009) and fish farming (Miriam furne *et.al*,2011). Starvation has certainly been a major factor in natural selection, since the beginning of life .The existing human and animal species is the result of the principle of survival of fittest which survived through countless episodes of food deprivation and shortage (Keyes *et.al*, 1950).When the crises of starvation hits the body of the fish, there seems to be differential utilization of three energy sources such as carbohydrates, proteins and also lipids so as to overcome the stressful situation. Starvation studies on some species of fish such as *Acipenser* and *Oncorynchus* , muscle protein was the last reserve the fish utilized (Navarro and Gutierrez,1995) and in some other species of fish proteins would be degraded for gluconeogenesis and lipid and or /protein being mobilized as energy substrates.(Sheridan and Mommsen,1991; Navarro and Gutierrez,1995; Gillis and Ballantyne,1996).Starvation studies on Penguins by Mario Spee *et.al*, (2011) have suggested the existence of three distinct phases during prolonged fasting characterized by lipid utilization during phase one, maintenance of constant rate of body mass and protein sparing in phase two and in phase three where animal depends on protein reserves. In another study by P.Vujonic *et.al,* (2011) on starving rats have also expressed a similar opinion stating that as and when the animal approaches the final phase of starvation, it is the proteolytic phase and is marked by simultaneous increase of both blood urea and corticosterone (CORT).A recent study on *Channapunctatus* (Sunnap Namrate *et.al*, 2011) has demonstrated a marked depletion in the protein content in fish which was subjected to starvation stress.

Based upon these studies, it is quite evident that proteins are known to play an important role in satisfying the energy demands of the starving animal .The present study is an attempt to understand if proteins were the preferred fuel over carbohydrates and lipids for *Anabas* during short-term and long term food deprivation. In this context we selected six tissues such as liver, kidney, brain, accessory respiratory organ pectoral and lateral line muscle so as to know if there was a tissue based selective mobilization of proteins during food deprivation.

MATERIAL AND METHODS

Fish weighing of 20-25gm were obtained from Kolleru lake of Eluru. Care was taken to ensure quick transport to the laboratory. Overcrowding was avoided during packing to minimize the mortality rate. They were carefully transferred into Durex storage tank of capacity 500 lits. made of material corrosive resistant polyproplene. The closed plastic lid of the tank was replaced by a grill lid made of iron. This helped in proper ventilation and aeration of the tank. Fish which were injured or dead were removed from the tank from time to time. Disinfectant ($KMnO_4$) was used to avoid infection. They were given boiled egg, rice bran meal and commercial fish feed *ad. libitum*. Any leftover feed and fecal matter were removed daily. Water in the tank was changed every day. Fish were brought to the laboratory and sufficient time was allowed for acclimatization. Experimentation was done thereafter. Fish measuring about 3'-4" in

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length and in the same range of weight were selected carefully. They were grouped together and kept in circular tubs made of plastic. The mouth of these tubs was covered with fine mesh and appropriately placed such that they were properly ventilated and well aerated. Two types of experimental set up were designed. In the first set up, fishes were allowed to starve for 15 days and parallel control was also maintained. The control animals were fed regularly both in the morning and evening. On the 16th day both experimental and control animals were sacrificed by concussion, and the tissues were removed for biochemical analysis. The II experimental set up consisted of fishes which were allowed to starve for 60 days (2 months; (long-term). Experimental group and a corresponding control group were maintained. Control group was fed regularly as in the case of short term. On the 61st day, the animals were sacrificed, for experimentation. Six animals from the control group and six from the experimental group were killed by concussion and the tissues were transferred on to ice blocks, weighed, homogenized, and experimented. The tissues selected for the experimentation were liver, kidney, brain, accessory respiratory organ, pectoral and, lateral line muscle.

Estimation of Proteins

Total protein content of the tissue was estimated by the method of Lowry, Farr and Randall (1951).

Procedure: Tissue homogenate was prepared with 10% TCA and centrifuged at 2000 rpm. for 15 minutes. Supernatant was discarded, while the precipitate was dissolved in 0.1 N NaOH(Sodium Hydroxide).To 1ml of sample, 4 ml of reagent "C" (alkaline copper solution) was added and allowed to stand for 10 minutes at room temperature. Then 0.4 ml of reagent "D" (Follin's reagent) was added rapidly with immediate shaking. After 30 minutes the colour intensity was read in a speckol colorimeter at 540 nm against the distilled water blank. The protein concentration was determined with the help of working standard prepared using albumin and expressed as milli grams of protein / gm wt. of tissue.

RESULTS

Short term starvation stress, for 15 days, lead to a significant depletion in protein levels of all the tissues, but for accessory respiratory organ which showed an increase in the protein content.

Liver showed a decrease of 35.6% (P<0.001). In kidney the decrease was 13.9% (P<0.001). Depletion in brain was 1.8% (NS). In pectoral muscle the decrease was 20.5% (P<0.001). Lateral line muscle showed a decrease of 20.1% (P<0.001) (Tables- I, II&III Figs: 1, 2&3).

Long term starvation stress for 60 days showed a decline in protein levels in tissues like kidney, pectoral muscle and lateral line muscle. Other tissues like liver, accessory respiratory organ and brain showed an elevation in the protein concentration. The decrease in kidney tissue was found to be 25.7% (P<0.001). Pectoral muscle showed a decline of 34.5% (P<0.001). The decrease in lateral line muscle was 27.7% (P<0.001). Liver showed an increase of 40.7% (P<0.01). The increase in brain tissue was 112.8% (P<0.001).Accessory respiratory organ showed an elevation of 1% (NS) (Tables-I, II, III Figs.-1, 2&3).

Table I. Total Protein Levels in Liver and Kidney tissues during Short Term and Long Term starvation in *A.testudineus*

S.No.	Tissue analyzed	Short Term (15 Days)		Long Term (60 Days)	
		Control	Expt.	Control	Expt.
1.	Liver	6.729 SE = ± 0.155	4.33** SE = ± 0.024	3.38 SE = ± 0.060	4.758* SE = ± 0.302
		% Variation = -35.651%		Variation = +40.769	
2.	Kidney	3.117 SE = ± 0.044	2.683** SE = ± 0.04	2.124 SE = ± 0.064	1.578** SE = ± 0.048
		% Variation = -13.923		% Variation = -25.706	

Values expressed in mg of protein / gm wt. of tissue
Each value is mean of SE ± of 6 individual observations
P < 0.01*, P < 0.001**

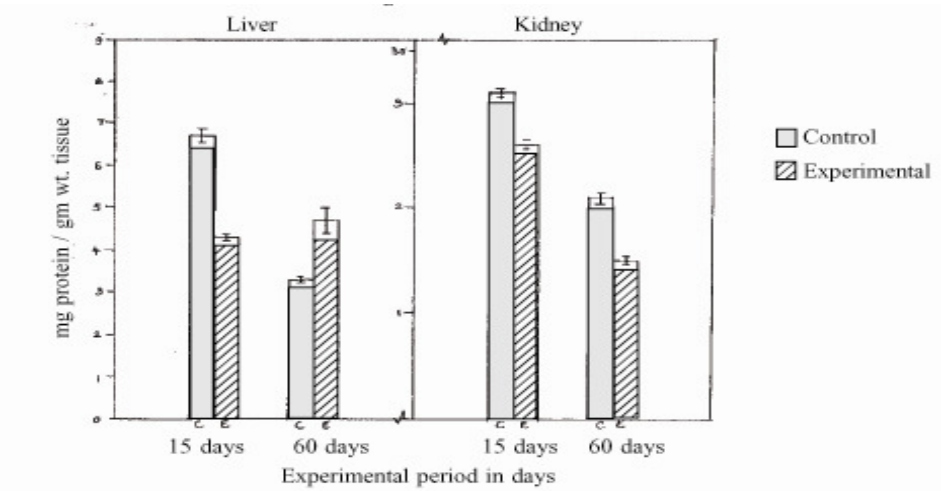

Fig 1.X axis: experimental Period in Days
Y axis: mg of protein/gm wt of tissue

Table II. Total Proteins Levels in Brain and Accessory Respiratory Organ during Short Term and Long Term starvation in *A.testudineus*

S.No	Tissue analysed	Short Term (15 Days)		Long Term (60 Days)	
		Control	Expt.	Control	Expt.
1.	Brain	2.269 SE = ± 0.051	NS 2.228 SE = ± 0.036	0.745 SE = ± 0.0469	1.586* SE = ± 0.0293
		% Variation = -1.805%		Variation = +112.885	
2.	Accessory Respiratory Organ	1.055 SE = ± 0.0316	1.353* SE = ± 0.0256	0.753 SE = ± 0.033	NS 0.761 SE = ± 0.024
		% Variation = +28.246		% Variation = +1.062	

Values expressed in mg of protein / gm wt. of tissue
Each value is mean of SE ± of 6 individual observations
P < 0.001*, NS = Not Significant

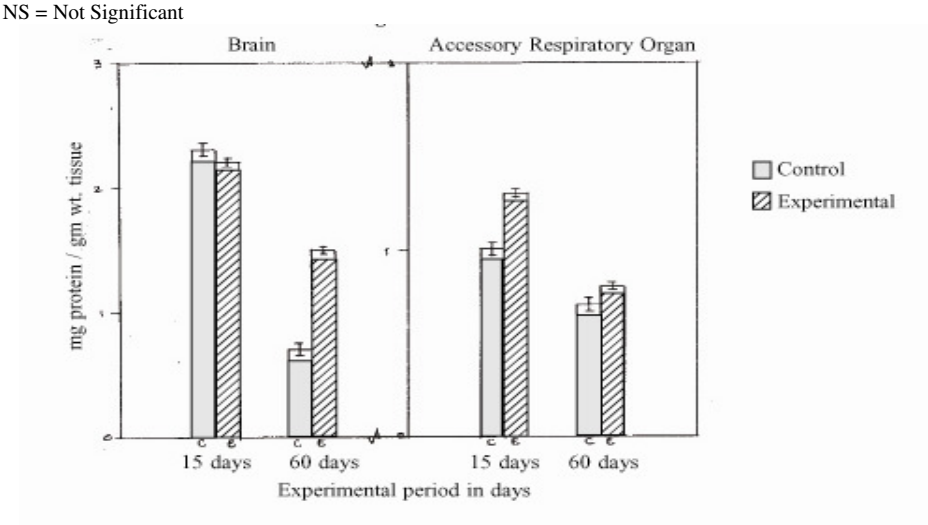

Fig 2.

Table III. Total Protein Levels in Pectoral and Lateral Line Muscles during , short Term and long Term starvation in *A.testudineus*

S.No.	Tissue analysed	Short Term (15 Days)		Long Term (60 Days)	
		Control	Expt.	Control	Expt.
1.	Pectoral Muscle	1.723 SE = ± 0.039	1.369*** SE = ± 0.003	1.297 SE = ± 0.028	0.849*** SE = ± 0.053
		% Variation = -20.545%	Variation = - 34.541		
2.	Lateral Line Muscle	1.681 SE = ± 0.045	1.343*** SE = ± 0.039	1.183 SE = ± 0.048	0.855*** SE = ± 0.046
		% Variation = -20.107	% Variation = -27.726		

Values expressed in mg of protein / gm wt. of tissue
Each value is mean of SE ± of 6 individual observations
P < 0.001***

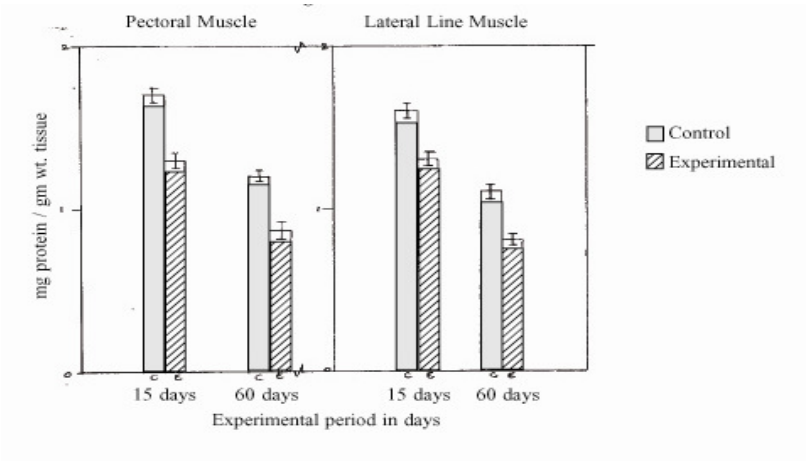

Fig 3.

DISCUSSION

In the present study we had observed an overall depletion in protein content during both the short-term and long term starvation regimes. Most of the tissues showed a significant decline in protein levels. Tissues such as kidney, and muscle (pectoral and lateral line) showed a significant depletion during both the fasting periods. Accessory respiratory organ showed an increase during both the periods of starvation stress. Strangely, liver and brain showed an increase in protein levels only during long-term starvation.

Liver: When *Anabas*, was subjected to short term fasting of 15 days, it resulted in the depletion of protein levels. This observation coincides with that of Dave *et.al* (1975), Larson and Lewender, 1973 and Collins and Anderson, 1995 who have all observed depletion in protein content during starvation in *Anguilla anguilla* and Golden perch respectively. Starvation studies by different authors such as Storer, (1967),Mann, (1972) Creach and Serfaty, (1974), Larson Lewander (1973),Shantha Vijaya Raghavan,(1988) have all observed an increase in amino acid degrading enzymes such as aminotransferase and asparartate aminotransferase and also elevation in gluconeogenic enzymes such as G6Pase,FDPase and PEPCK was observed by R.Premakumari,(1988) in her starvation experiments in *Anabas*. All of these observations point to the occurrence of gluconeogenesis so as to provide glucose to the starving animal and the precursors for this process are provided by the protein. Protein catabolism is of prime importance during fasting and conservation of amino acids for protein synthesis becomes secondary (Harper, 1965).In this context of gluconeogenesis, certain hormones such as cortisol, insulin and glucagon are known impact this process. It has been suggested that cortisol excess, insulin deficiency (Bellamy *et.al*, 1968; Exton, 1972) and altered balance between glucagon and insulin (Cahill, 1966) all are known to enhance gluconeogenesis. Apart from hormones, cyclic AMP is also known to have an impact on glycogenolysis and gluconeogenesis (Jefferson *et.al*, 1968).

With reference to hormones, insulin in particular, we (P.Godavarthy and Y.Sunila Kumari, 2011) have observed a significant decline in insulin, during both the fasting regimes and perhaps this insulin deficiency may have lead to enhanced gluconeogenesis resulting in decline in the protein content in liver so as to satisfy the energy demands of the starving animal.

However during the long-term food deprivation, the results were different and strangely showed an elevation in protein content .This in accordance with the observations of Bouche and Villas (1975) on starving *Cyprinus carpio*, which showed a protein elevation, and suggested a simultaneous synthesis occurring along degradation. An increase in protein content during starvation may also be due to conservation of proteins taking place and the starving animal surviving at the expense of glycogen as suggested by Kamara, (1966),Inui and Oshima, (1966) and Patent, (1970), Kerr *et.al*, (1978).The increase in protein concentration in the present study may be partially due to conservation of protein and the fasting *Anabas*, deriving its energy from other energy reserves such as glycogen or lipid or a simultaneous synthesis of proteins occurring along degradtaion such as storage proteins which may be needed for gonad maturation as suggested by Brad ford, (1993) and Iles, (1984) in Atlantic herring.

Kidney: Brief and prolonged fasting of *Anabas*, showed a significant depletion in proteins in this tissue. Similar observations have been reported by Premakumari (1988). Her studies on *Anabas*, revealed high rates of protein catabolism with subsequent rise in the amino acid levels. It has been suggested by Exton, (1972) and Krebs (1964) that during prolonged starvation renal gluconeogenesis increases and hepatic gluconeogenesis decreases.

So it may be said that the decline of protein levels in this tissue may be attributed to enhanced gluconeogenesis and thus supplying energy to the starving animal.

Brain: In this tissue during brief fasting (of 15days) we observed, an insignificant decline in the protein levels. However the long term (60days) starvation showed an increase in the protein levels.

Short-term fasting in *Anabas*, led to a significant depletion in the protein levels of brain. This depletion may be attributed to their utilization for the metabolic activity of the brain. According to the studies of Walton and Cowey, (1982) body proteins in fish are in a continual state of turnover, being broken down and re synthesised in varying degrees. The depleted protein levels indicate protein utilization as brain is known to contain all enzymes of major metabolic pathways (Abood *et.al*, (1952); Berl *et.al,* (1962).

Long-term fasting showed an increase which may be explained as a simultaneous synthesis taking place along degradation as suggested by Walton and Cowey,1982. The synthesis of proteins may be for the synthesis of certain important enzymes like glutamine synthetase and glutamate decarboxylase. Glutamine synthetase is known to play a protective role in fish brain and Walton and Cowey, (1982).

Accessory Respiratory Organ: The results from this tissue are surprising, as we have observed an increase both the periods of starvation. The present starvation study on *Anabas*, resulted in elevated levels of proteins, which was not very significant. This slight increase in the protein levels may be due to slow mobilization of proteins. It may also be said that there may be also a synthesis of proteins occurring along their degradation. The enzymes required for amino acid catabolism may be synthesized during starvation. Since enzymes are also proteins, hence there is an elevation in the protein level in this tissue.

Muscle: Both the pectoral and lateral line muscle showed very significant decline in the protein levels during both the fasting regimes. These results coincide with that of Lim and Ip (1989) who have studied the effect of fasting on glycogen metabolism and various glycolytic and gluconeogenic enzymes in the mudskipper (*Boleophthalmus boddaerti*) and observed a reduction in muscle protein. Similarly, protein depletion is *Anabas* during food deprivation suggests its utilization for energy needs as protein is known to be the primary energy source during starvation as seen in most of the fish species (Cahn and Woo, 1978a,Larson and lewander,1973 and Lorven, 1939,Creach and Serfaty,1974).Starvation causes utilization of proteins *via* gluconeogenesis after being mobilized to gluconeogenic organs,((Moon,and Johnson, 1980; Black and Love,1986 and Sheridan and Momeson,1991), and both of these processes are influenced by hormones such as glucocorticoids (Cannon *et.al,* 1956)

CONCLUSIONS

The present study revealed a non uniform depletion in certain tissues and in some tissues a mild elevation in protein content. There was a tissue based selectivity observed in the mobilization and utilization of proteins. In conclusion it may be said that during the short-term and long-term starvation stress of *Anabas*, proteins are being utilized *via* gluconeogenesis after being mobilized to gluconeogenic organs along with other energy sources such as glycogen and lipids. There may be enhanced gluconeogenesis occurring due to insulin deficiency and cortisol excess. The mild increase in proteins in some tissues may be attributed to a simultaneous synthesis of proteins occurring along degradation which may be to produce certain protective enzymes or proteins needed for gonad maturation.

REFERENCES

[1] Lowry, O.H., Farr, A.L. and Randall, R.J.1951"Protein estimation with Folin ciocaltin Reagent." *J. Biol. Chem.* 193: 265.

[2] Larsson Ake and Lewander Kerstin ; 1973"Metabolic effects of starvation in the eel, Anguilla Anguilla L." *Comp. Biochem. Physiol*. Vol.44A pp.367-374.

[3] Loughna, P.T. and Goldspink, G.1984"The effects of starvation upon protein turnover in red and white myotomal muscle of the rainbow trout *Salmo gairdneri* Richardson. *Journal of Fish Biology 25, 223-230.*

[4] Machado, C.R., Garofalo, M.A.R., Roselino, J.E.S., Kettelhut, I.C. & Migliorh R.H. 1988 "Effects of Starvation, refeeding and insulin on energy linked metabolic processes in cat fish (*Rhamdia hilarii*) adapted to a carbohydrate rich diet. *General and Comparative Endocrinology. 71, 429-437.*

[5] Hinch,S.G., Cooke,s.j., Haeley,M.C and Farrel,A.P.(2005)."Behavioral Physiology of fish migrations": Salmon as a model approach, *Fish Physiology 24:239-295*

[6] Miller,K.M., Schulze, A.D., Ginther,n.liS., Ptterson,D.A., Farell,A.p. and Hinch, S.G (2009).Salmon spawning migrations: metabolic shifts and environmaental triggers, *Comp Biochem physiol 4* d:75-89

[7] Miriamfurne,amalia Morales, E., Cristine, E.Trenzado,Manuel,graciagallego.M.carmen Hidalgo and AlbertoDomenzain.AnaSanzrus,(2011)."The metabolic effects of prolonged starvation and refeeding in sturgeon and rainbow trout. *J.Comparative Physiology B*

[8] Keys Ancel Josef Brozek, Austin Henschel, Olaf Mickelsen Henry Longsteet Taylor. 1950 The Biology of Human Starvation Vol.I,Vol.II. The University of Minnesota Press Minneapolis, London, Geoffrey Cumberlege. Oxford University Press, 289-300; 575,576.

[9] Navarro I., Gutiererez.J., 1995; fasting and starvation In: Hochachaka,P.W,Mommesen TP(eds) Biochemistry and Molecular biology of fishes, metabolic biochemistry vol:4,Elsevier,Amsterdam pp-393-434

[10] Sheridan, M.A. and Mommsen, T.P.1991"Effects of nutritional status on in *vivo* lipid and carbohydrate metabolism of Coho Salmon, *Oncorhynchus Kisutch.*" *General and Comparative Endocrinology. 81, 473-483.*

[11] Gillis T E, Ballantyne J.S ,1996 "The effects of starvation on plasma free aminoacid and glucose concentrations in lake sturgeon, *Acipenser fulvescens*; *J.fish Biol 49: 1306-1316*

[12] MarioSpee,Lorene marchal,Anne-Mathilde Thierry,Oliver chastel, Manfred enstipp,Yvon Le Maho, MiCahel Beaulieu and Thierry Rachlot,2011" Exogenous Corticosterone mimics a late fasting stage in captive adelie Penguins (*Pygoscelis adeliae*) *Am J.Phsiol.Regul.IntegrCompPhysiol.300:R1241-1249*

[13] P.Vujovic,I.Lakic,D.Laketa,N.Jasmic,S.F,Djurasevic,G.Cvijic,J.Dj ordjevic,2011 Time dependent effects of starvation on serum, Pituitary and Hypothalamic leptin levels in Rats" *Physiol.Res 60(Suppl.I) S165-S170*

[14] Sunnap Namrata, Nanware Sanjayand Chavan Pallavi,2011 "Effect of Starvation on biochemical Composition of freshwater fish "*Channa punctatus; Recent Research in Science and technology , 3(9), 17-19*

[15] Dave Goran, Maj-Lis Johansson - Sjobeck, Ake Larsson, Kerstin Lewander and Ulf Lidman.1975;"Metabolic and Haematological effects of starvation in the European EeL, Anguilla Anguilla L. - I. Carbohydrate, Lipid Protein and inorganic ion metabolism. *Comp. Biochem. Physiology. Vol.52A. pp.423 to 430.*

[16] Collins, A.L. and J.A. Anderson.1995 "The Regulation of endogenous energy stores during starvation and refeeding in the somatic tissues of golden perch." *J. of Fish Biology. 47, 1004-1015.*

[17] Storer, J.H.1967 "Starvation and the effects of cortisol in the gold fish (*Carassius auratus L.) Comp. Biochem. Physiol. 20, 939-948.*

[18] Mann, O.E. 1972 "B.Sc.Thesis, New Brunswick (cited by Whiting & Wiggs).

[19] Creach and Serfaty, A.1974"Lejeune et la realimentation chezla carpe." *J. Physiol*. Paris. 68, 245-260.

[20] Shanta Vijayaraghavan. 1981"Some aspects of physiology of digestion in fish." Ph.D. Thesis, Osmania University, Hyderabad. (India).

[21] Premakumari, R. (Thesis) 1988"Some Aspects of Physiology of fish under nutritional stress." Osmania University, Hyderabad, A.P., India.

[22] Harper E. Alfred 1965"Effect of variations in Protein intake on Enzymes of Amino Acid Metabolism." *Canadian Journal of Biochemistry. Vol.43. 1589-1603*

[23] Bellamy, D., Leonard R.A. and Dulien K.1968"Hepatic gluconeogenesis in rats treated with cortisol." *Gen and Compar. Endocr. 10, 434-437.*

[24] Exton, J.H.1972 Progress in Endocrinology & Metabolism "Gluconeogenesis" Metabolism - Vol.21, No.10, P.No.945-990.

[25] Cahill, G.F. Jr., Herrera, M.G., Morgan, A.P., Soeldner, J.S., Sheinke, J., Levy, P.L.Reichard G.A., Jr, and Kipnis, D.M.1966"Hormone fuel Interrelationships during fasting." *J. of Clin. Invest. 45: 1751.*

[26] Jefferson, L.S., Exton, J.H., Butcher, R.W., Sutherland, E.W. and Park, C.R.1968"Role of adenosine -3', 5'-monophosphate in the

effects of insulin and anti-insulin serum on liver metabolism." *J. Biol. Chem. 243: 1031.*

[27] P.Godavarthy and Y.Sunila Kumari, 2011 "An Investigation of Plasma insulin levels during starvation stress in *Anabas testudineus* (Bloch) *Bulletin of Pure and applied sciences, vol-30A,INSN No:2*

[28] Bouche, G and Vellas, F.1975 "The speeds of renewal of the proteins of liver, muscle and plasma in carp (*Cyprinus Carpio*) submitted to total and prolonged starvation. *Comp. Biochem. Physiol. 51A, 185-193. 196, 197.*

[29] Kamra, S.K.1966 "Effect of starvation and refeeding on some liver and blood constituents of Atlantic Cod. (*Gadus moruhua L.*) *J. Fish. Res. Bd Can. 23, 975-982.*

[30] Inui, Y. and Oshima, Y.1966 "Effect of starvation on metabolism and chemical composition of eels." *Bull. Jap. Soc. Scient. Fish. 32, 494-501.*

[31] Patent, G.J.1970"Comparison of some hormonal effects on carbohydrate metabolism in an elasmobranch (*Squalus acanthias)* and a holocephalan (*Hydrolagus Colliei) Gen.Comp. Endocr. 14, 215-242.*

[32] Kerr S. Douglas, Michael C.G. Stevens, and Hazel M. Robinson.1978 "Fasting Metabolism in Infants. I. Effect of severe undernutrition on energy and protein utilization." Metabolism, vol.27, No.4. P.No.411-434.

[33] Bradford, R.G.1993"Differential utilization of storage lipids and storage proteins by Northwest Atlantic herring (*Clupea harengus harengus*). *Journal of Fish Biology. 43, 811-824.*

[34] Iles, T.D.1984"Allocation of resources to gonad and soma in Atlantic herring, *Clupea harengus* L. In Fish Reproduction (Potts. G.W.& Woolton, R.J., eds) London: Academic Press.

[35] Krebs, H.A. The Croonian Lecture - 1964 Gluconeogenesis. By Sir Hans Krebs. 1964Proceedings of the Royal Society of London Series -B. Vol.159, pp.545-564.

[36] Walton, M.J. and Cowey, C.B.1979 Gluconeogenesis by isolated hepatocytes from rainbow trout. *Comp.J Biochem. Physiology. 62B, 75-79.*

[37] Abood, L.G.R.W. Gerard, J. Banks, and R.D. Tschirgi. 1952 "Substrate\and Enzyme Distribution in Cells and Cell fractions of the Nervous System." *Am. J. Physiology. 168: 728.*

[38] Berl, S.G., Takagaki, D.D. Clarke and H. Waelsch. 1962"Carbondioxide Fixation in the brain." *J. Biological Chemistry. 237: 2570.*

[39] Lim, A.L.L. and Ip, Y.K.1989"Effect of fasting on glycogen metabolism and activities of glycolytic and gluconeogenic enzymes in the mudskipper *Boleophthalmus boddaerti." J. Fish. Biology. 34, 349-367*

[40] Chan, D.K.O and Woo.1978a"Effect of hypophysectomy on the chemical composition and intermediary metabolism of the Japanese eel." *Anguilla japonica. Gen. Comp. Endocrinol. 35, 169-178.*

[41] Lovern, J.A.1939"Captive eels. Some observations on their behaviour." Salmon & Trout Mag. 94, 56-57.

[42] Creach and Serfaty, A.1974 "Lejeune et la realimentation chezla carpe." *J. Physiol. Paris. 68, 245-260.*

[43] Moon, T.W. and Johnston, I.A.1980 "Starvation and the activities of glycolytic and gluconeogenic enzymes in skeletal muscles and liver of plaice." *J.Comp.Physiology. 136B, 31-38.*

[44] Black, D and Love, M.R.1986"The sequential mobilization and restoration of energy reserves in the tissues of Atlantic Cod during starvation and refeeding*." Journal of comparative Physiology. 156B, 469-479.*

[45] Sheridan, M.A. and Mommsen, T.P.1991"Effects of nutritional status on in vivo lipid and carbohydrate metabolism of Coho Salmon, *Oncorhynchus Kisutch." General and Comparative Endocrinology. 81, 473-483.*

[46] Cannon, P.R., Frazier, L.E. and Hughes, R.H.1956 "The influence of cortisone upon protein metabolism." *Archs. Path. 61, 271-279.*