Proceedings of the Summer Institute in Recent Advances in Finfish and Shellfish Nutrition

11 TO 30 MAY 1987

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
Dr. SALIM ALI ROAD
COCHIN-682 031

Technical Paper No.5

SUMMER INSTITUTE IN
RECENT ADVANCES IN FINFISH AND SHELLFISH NUTRITION

11-30 May, 1987

PROTEIN AND AMINO-ACID REQUIREMENTS IN FINFISHES AND SHELLFISHES

D.C.V. EASTERSON

Tuticorin Research Centre of
Central Marine Fisheries Research Institute,
Tuticorin-628 001.

Proteins are macromolecules, which are biopolymers made up of many monomers which are known as amino acids. Of the three carbohydrate, lipid and protein, it is only proteins which contain nitrogen. The empherical formula for amino acid is R-CH-NH_2-COOH. Though about 300 or so amino acids are known to occur in nature, only 20 of these are present in proteins and all of them are L-α, amino acids. The sequence in which these amino acids occur in a particular protein follows a precise order, which is genetically controlled. Thus each peptide molecule ie., protein differs from another only by the order of arrangement and in the number of amino acid molecules.

FUNCTIONS OF PROTEINS

Proteins are vital as are the functions they perform. The functions they perform either as pure proteins or as complexes with carbohydrate, lipids, and minerals are many. Growth of the animals is nothing but addition of tissues ie., synthesis of new protein. Thus as structural proteins are responsible for the cellular architecture. The other functions are (2) in the body fluids they transport

substrates; (3) several of the hormones and (4) enzymes which catalyse biochemical reactions are proteins; (5) proteins form component in immunologic molecules; (6) serve as lubricants and protective agents (mucins, mucos); (7) the antifreeze substances in the Antartic fishes are glycoproteins; (8) many of the toxins and venoms of marine organisms are protein complexes; (9) some of the amino acids have been found to be feed attractants; (10) protein molecules also have a high buffering capacity; (11) glucogenic - amino acids (hydroxyproline, serine, cysteine, threonine, glycine; tryptophan, alanine; tyrosine, phenylalanine; isoleucine, methionine, valine; histidine, proline, glutamine, arginine, glutamate; and aspartate) on being deaminated serve as substrates for carbohydrate and fatty acid synthesis (Gluconeogenesis) and (12) thus also yield energy.

CALORIE VERSUS PROTEIN AS UNIT OF MEASUREMENT IN NUTRITIONAL BIOENERGETICS

In the study of energetics energy in terms as calories or joules used to be preferred. But for aquatic organisms partitioning based on protein as nitrogen units is of more suitable over energy units for the following reasons: 1. In comparison with higher animals fin - and shell-fishes being poikilotherms use less energy to regulate their body temperature. 2. For the locomotion and maintenance of position, shellfishes need not spend much energy being mainly bottom dwellers for much of the time. 3. The shellfishes for the purpose of respiration like fishes need not actively maintain ventilation of gills by constant flow of water which in turn compels active swimming, a costly process in terms of energy. 4. In finfishes and in

shellfishes it is an important aspect that protein serves not only as a nutrient for growth but preferred over carbohydrates as dietary energy source. (5) The quantity assimilated over maintenance level in carbohydrate and lipid is stored mostly as fat and as glycogen to a lesser extent; while in the case of proteins goes for meat production. Many consumers do not like fatty aquatic products. (6) The end product of nitrogenous metabolism in the aquatic organisms is mostly ammonia which by passive diffusion can be eleminated into the medium (7) Thus energy need not be spend in converting the toxic ammonia into urea or uric acid, whereby aquatic organisms come to derive more metabolisable energy from catabolism of proteins than terrestrial organisms. To illustrate: for a megajoule of digestible energy in rainbow trout (Salmo gairdneri) 9.6 g body protein is produced which is 2 to 20 times higher for poultry, pig and cattle (Pandian and Vivekanandan, 1985).

ESTIMATION OF PROTEIN

Protein is usually estimated by any one of the following methods. (1) Determination of nitrogen by kjeldahl method; (2) Biuret method and (3) Folin-Lowry method. Of these the first given method is mostly used, where in all nitrogenous matter-both protenous and nonprotenous-is converted into ammonia and calculated in terms nitrogen. (When protein alone need to be determined first protein need to be precipitated out and precipitate is digested for kjeldahl nitrogen). In the conversion of nitrogen into crude protein it is assumed that all nitrogen in the biological material is present as protein and secondly that all crude proteins contain nitrogen 16% by weight and so the conversion factor used is 6.25 (100/16=6.25). This is not always so. Therefore check need to be made for percentage

Soyabean meal: CP 39-41% in dry matter, crude fibre 9%, availability of amino acids is high (CS 82-92%) except for methionine (CS 70%). However heat treatment used to inhibit typsin inhibitor reduces the availability of lysine and cystine, much in the case other amino acids too is reduced. Raw meal causes rachitogenic effect. Therefore higher than normal levels of vitamin D_3 need to be added. It is also suggested to have tocopherol oxidase. Vitamin content too get reduced with heat treatment.

Wheat (Triticum aestivum) : CP 6-22%, average 8-14%, protein (gluten) is of two types. (i) Prolamin (gliadin) and (ii) Glutelin (glutenin). The second contains three times more lysine than the first. Gluten is rich in glutamic acid (33%) and proline (12%).

FORMULAE AND INDICES USED

The indices used in the measurement of protein utilisation are as follows:

1. Assimilability (digestibility) of protein (or assimilation efficiency of protein) A%

$$= \frac{\text{Protein consumed(g) - faecal protein (g)}}{\text{Protein consumed (g)}} \times 100$$

2. Nitrogen balance (NB) = N consumed -(N in faeces+ N excreted through gills and kidney)

NB is measured in terms of mg N/100g body weight/day. Therefore all the 4 parameters need to be in the same unit.

3. Protein efficiency ratio (PER)

$$= \frac{\text{gain live weight (g)}}{\text{protein consumed (g)}}$$

4. Protein conversion ratio (PCR) = $\frac{\text{Protein gained (g)}}{\text{Protein consumed (g)}}$

5. Net protein retention (NPR) = $\frac{\text{Weight gain of TPG(g) + weight loss of PFG (g)}}{\text{Protein consumed (g)}}$

TPG - group fed with test protein

PFG - group fed with protein-free diet

6. Productive protein value (PPV) (%) = $\frac{\text{gain in body protein(g)}}{\text{protein consumed (g)}} \times 100$

7. Meat produced in assimilation (MPA) (%) = $\frac{\text{live weight gain (g)}}{\text{protein assimilated(g)}} \times 100$

8. Protein produced in assimilated protein (PAP)(%) = $\frac{\text{Protein gained (g)}}{\text{Protein assimilated(g)}} \times 100$

9. Gross protein value (GPV) = $\frac{A}{A_o} \times 100$

A = (weight gain of Gr. 2 - that of Gr. 1) $\div$ weight gain of Gr. 2

A_o = (weight gain of Gr. 3 - that of Gr. 1) $\div$ weight gain of Gr. 3

Diet groups:

Group 1:- fed with basal diet

Group 2:- fed with basal diet + Cg of test protein

Group 3:- fed with basal diet + Cg of casein

Basal diet will be having optimal crude protein

10. Apparent biological value (ABV) (%) = $\frac{\text{N consumed - (faecal N + urinary N)}}{\text{N consumed - faecal N}} \times 100$

11. Biological value (BV)(%) $= \dfrac{\text{N consumed} - [(\text{faecal N} - \text{MFN}) + (\text{urinary N} - \text{EUN})]}{\text{N consumed} - (\text{faecal N} - \text{MFN})} \times 100$

MFN - Metabolic faecal nitrogen is that quality of nitrogen excreted in the faeces when the animal is fed with nitrogen free diet.

EUN - Endogenous urinary nitrogen is that quantity of nitrogen excreted by means of gills and as urine when the animal is starved of dietary protein.

12. Daily protein requirement (% live weight/day) = (Optimal dietary protein requirement % x consumption of feed % live wt per day) $\div$ 100

Instead of 100, if divided by 10 will give protein required for kg live weight per day.

13. Protein required for weight gain (g/kg live wt.) = Optimal dietary protein requirement (%) X FCR x 10

Where FCR (food consumption rate) = food consumed $\div$ weight gained (dry weight basis)

14. Chemical score: The quality of protein in a protein source is decided by the quantity of EAA present. Here EAA content of the source is compared with that of a standard protein.

The usual standard used by the nutritionists in hen's egg white. The current trend with the Japanese workers particularly with Ogino group is to use the EAA profile of the fish muscle as standard protein in the fish nutrition. CS is calculated as follows.

Eg: Tryptophan in egg white - 1.7%

Tryptophan in sardine - 1.2%

$$CS = \frac{1.2}{1.7} \times 100 = 70.59\%$$

15. EAA index : Herein the amounts of all the 10 essential amino acids present are taken into consideration. It could be defined as the geometric mean of egg ratios of these acids.

$$EAAI = \sqrt[n]{\frac{100_a}{a_e} \times \frac{100_b}{b_e} \times \frac{100_c}{c_e} \cdots \frac{100_j}{j_e}}$$

a, b, --- j = % EAA in the protein source

a_e, b_e -- j_e = % in the egg albumin

n = number of EAA entering into the calculation

EAAI has the advantage of predicting the effects of supplementation in combination of proteins but proteins of very different EAA composition may come to have a similar index.

OPTIMAL DIETARY PROTEIN REQUIREMENT

Organisms need to be supplied with sufficient quantity of protein in their diet for their metabolic needs and growth. Protein is a costly commodity and so it is protein which is the single major component which decides the price of the feed. When higher levels of protein is available in the feed some portion of it will go waste. Thus protein need to be at an optimal level in the feed. While conducting experiments to arrive at the optimal dietary protein requirement the following points need to be carefully considered. Foremostly the protein which needs to be evaluated should be sufficiently able to meet the requirement for essential amino acids. If one essential amino acid is deficient while all the other amino acids are available in enough quanity, complete spectrum of protein synthesis can not be met. Usually for the purpose purified proteins such as casein, albumin or mixture of proteins are used. Casein is

deficient in argenine and suboptimum in sulphur bearing aminoacids, zein is deficient in tryptophan and lysine. In such cases these essential amino acids need to be supplemented. Usually for the purpose crystalline amino acids are used. It has been found that free crystalline amino acids are not so well absorbed as that of bound amino acids in fishes. Regarding other marine organisms still we do not know how far free amino acids are absorbed (Jacon and Cowey, 1985). It is also to be noted that carnivorous fishess show low palatability of purified proteins.

Second point to be observed is that the level of feeding should not be a constraint for optimal growth. Ad libitum feeding is recommended. Another fact which need to be emphasised is, the feeding strategy should be convenient to the test animal. By feeding strategy, suitability of the feed for the animal's style of foraging, particle size of feed, time, frequency and duration of feeding, form of feed whether pellet, powder or paste etc., and ecophysical conditions of like light, salinity, pH, temperature, vibration and disturbance to the animal etc., are meant.

When the values of indices like K_2, EMP, MPA, PAP, PER, PCR and PPV are plotted against % protein in diet at the optimal protein level the graph will peak, whereby indicating the optimal requirement. At lower levels the assimilation of the feed and protein have been found to be high while low at higher levels. Consumption of the feed has been found to be high in low protein diet and also when the protein is found to be low in one or more EAA, whereby the organism attempts to gather more of the required nutrient. In such cases protein assimilation and consumption rate have been found to be high; while other nutrients available at optimum concentration in the diet are preferentially assimilated less. In crustaceans it has been observed that at

high (about 60%) protein level too feed consumption show a rise. In such cases assimilation for all nutrients is low. Thus the animal used to take to superfluous feeding also known as "gluten effect".

Though weight gain is used by some workers in the interpretation of data, gain in protein (protein retention) or nitrogen balance is preferred. In crustaceans the interpretation of data pose characteristic situation because of moulting. The animals just moulted used to be flaby and high in water content. If such animals happened to be there at the conclusion of the experiment indices in which live weight is taken into calculation can be misleading.

A survey of literature show that optimal dietary protein requirement ranges between 36-50% (Table 1). Though there are a few rare instances where in value as high as 70% has been quoted. The average is around 39% for finfishes and shellfishes. While getting high percentage it is noteworthy to note that EAA deficient protein can elevate dietary protein requirement. Further increase in water temperature above ambient upto an optimal level increases dietary protein requirement. In this connection it need to be pointed out it is well known that increase in water temperature upto an optimum is accompanied by an increase in feed intake coupled with higher metabolic rate and increased growth. Whereby tropical organism have higher feed intake level coupled with faster growth rate.

DAILY PROTEIN REQUIREMENT

Comparatively very few investigations alone have been carried out in this direction. The available data indicate that daily protein requirement does not fall within a narrow

range as do optimum protein requirement discussed above. In fishes it ranges from 0.75 to 5.25 in terms of percentage body weight per day. The interesting fact is that a linear relationship exists between daily protein requirement and the specific growth rate. (Jacon and Cowey, 1985). Thus it is amply clear that optimal dietary protein requirement and daily protein requirement are not related factors. The optimum dietary protein requirement is related to concentration vs activity, ie., quantity required for the optimum rate of digestion and assimilation. While daily requirement is related to the inherent capacity of the animal to grow in other words to the speed of protein synthesis, which is dependent on metabolic activity, age, size, temperature and hormonal control.

REQUIREMENT OF ESSENTIAL AMINO ACIDS

Essential amino acids are those amino acids which cannot be biosynthesised by the organism sufficiently. It is of interest to note that essentiality for 10 amino acids seems to be universal throughout the metazoa, though a few variation from the general pattern is met. The essential amino acids are - threonine, valine, methionine (+ cystine), isoleucine, leucine, phenylalanine (+ tyrosine), tryptophan, lysine, histidine and arginine. Tyrosine, cystine, glycine and serine could not be synthesised by the organism in sufficient level and so need to be supplied in a lesser extent therefore known as semiessential amino acids. Glutamic, aspartic acids, alanine, proline and hydroxyproline are non essential amino acids. Since there can be synthesised in required level. The figure given on the inter-conversion of the major food stuffs amply illustrate the synthetic and interconversion of non essential amino acids. Though cystine from methionine and tyrosine from phenylalanine could be

synthesised, in the absence of cystine and tyrosine the requirement for methionine and phenylalanine is increased.

The synthetic pathways of semi and non-essential aminoacids are as follows. (Fig. 1 & 2).

Alanine	By transamination of pyruvate with glutamate.
Aspartate	By transamination of oxalacetate with glutamate.
Proline	From glutamate via glutamate semialdehyde and pyrroline carboxylate.
Glutamate	By reductive amination of - Ketoglutarate
Arginine	By reactions of urea synthesis
Glycine	By removal of hydroxymethyl group from serine
Serine	By transamination of hydroxypyruvate or phosphohychoxypyruvate with alanine.
Tyrosine	From phenylalanise by hydroxylation
Cystine, Cysteine & Taurine	By transulfuration pathway from methionine

Thus theoretically all non-EAA except the sulphur bearing (Cystine, Cysteine, Taurine) can be synthesised in the organism by feeding sufficient ammonium salts together with glucose to provide the carbon skeleton.

METHODS OF DETERMINATION

The following methods have been used to evaluate EAA requirements.

I. Direct method: Herein as one at a time basis each aminoacid is deleted from the amino acid test diet and a dose-response growth curve is made. Dietary requirement is taken at the 'break - point'.

II. Indirect methods:

2. Nitrogen balance technique: This is a modified method of the first one. Herein quantitative variation in free amino acid levels in specific tissue pools such as, whole blood, plasma, haemolymph or muscle is made with reference to the deleted amino acid on enquiry.

3. Tissue culture method: like dietary deletion herein specific amino acid free media are used.

4. In alternatively starved and fed animals fluctuations in of free amino acids levels are made in tissue pools; wherein EAA fluctuate drastically between feeding and starvation while the levels of non-essential amino acids remain steady.

5. Radioisotopic assay: Animal is fed or injected with one of the radioactivity labelled readily metabolisable metabolite such as (^{14}C) glucose; $^{14}CO_2$, (^{14}C) acetate, (^{14}C) succinate or (^{14}C) pyruvate. Organism (if small) or a part of the tissue is latter assayed after a period of incubation. The non-essential amino acids being able to be synthesised from the precursors take up labelling while EAA remain unlabelled. Since many of the microbes have the capacity to synthesis EAA, in this method microbiological contamination is the chief source of error. It has been found out that molluscs in general have a strong capacity for a rapid biosynthesis of glutamate, alanine, and aspartate and weaker or non-existing capacity for asperagine, glutamine, serine, glycine and proline from glucose moity. Aspartate is most strongly labelled with succinate and CO_2 precursors; while alanine with glucose and pyruvate. Among aspartate, alanine and glutamate, glutamate is generally

most weakly labelled. These indicate a considerable capacity for CO_2 fixation into dicarboxylic acids of TCA cycle and a tendency for many of the molluscs to accumulate alanine under anaerobic conditions (Bishop et al., 1983).

6. Ogino's carcass deposition method (Ogino, 1980a&b): This is the only method devised to determine quantitative requirement for EAA specifically for fishes. Ogino observed similarity in percentage composition existing between dietary EAA requirements of fishes and EAA profile of fish muscle. Since the crustalline amino acids have been found not so ideal as sources for EAA he preferred lipid free fish meal or lipid free fish muscle as dietary protein source ie., standard essential amino acid reference dietary protein. His procedure is to estimate daily nitrogen/protein retention rate, percentage feeding rate for 100 g body weight, percentage digestibility for protein and for each amino acid for the test animal. From these parameters he calculated optimum level for each amino acid required to be present in the dietary protein source and optimum dietary requirement per day for each amino acid. Select list of EAA requirement to some of the cultivable animals is given in the Table 2.

Comments:

The EAA study carried out in Mytilus californianus (Harrison, 1980) show that apart from known ten EAA, proline is also essential. In eel cystine is superior to methionine, while in other animals it is other way. In eel cystine at the rate of 0.05% and methionine at 1.6% in the diet failed to premate growth while at 1.0% and 0.9% levels respectively resulted in enhanced growth.

Many lower marine organisms, especially marine molluscs have ability to absorb all protein amino acids including

taurine from the medium. Only marine molluscs require taurine a non EAA, while freshwater and terrestrial molluscs do not. The uptake of glycine, threonine and glutamin was very fast from the medium while arginine was taken up slowly. The studies show that gills are the main organ of absorbance. The interesting finding is that there are specific transportation site for each group of amino acids, viz., acidic, basic, neutral and imino amino acids. Even dipeptides have been found to be absorbed. Transportation of alanine, glycin and cycloleucine has been found to be either sodium ion and/or energy dependent (Bishop et al., 1983).

In rainbow trout tryptophan deficiency has been known to induce loss of appetite, transient scoliosis and deposition of calcium in bony plates around notochord and kidney. The fish also becomes hyperemic (Cowey and Sargent, 1979).

LITERATURE CITED

Bishop, S.H., L.L. Ellis and J.M. Burcham 1983. Amino acid metabolism in molluscs. In Mollusca Vol. 1. Metabolic Biochemistry and molecular Biomechanics, pp. 243-327, Academic Press.

Cowey, C.B. and J.R. Sargent 1979. Nutrition. In: Fish Physiology Vol.VIII, Hoar, W.S., D.J. Randall and J.R. Butt (Eds), pp. 1-69, Academic Press.

Harrison, C. 1979. Essential amino acids for Mytilus californianus. Veliger, 18(2): 189-193.

Jacon, A.G.J. and C.B. Cowey 1985. Protein and amino acid requirements. In: Fish Energetics: New Perspectives, Tytler, P. and P. Calow (Eds.) pp. 155-183, Croom Helm.

Ogino, C. 1980. Protein requirements of carp and rainbow trout. Bull. Japan Soc. Sci. Fish., 46: 385-389.

Ogino, C. 1980. Requirement of carp and rainbow trout for essential amino acids. Ibid., 46: 171-176.

Pandian, T.J and E. Vivekanandan 1985. Energetics of feeding and digestion. In: Fish Energetics: New Perspectives, Tytler, P and P. Calow (Eds.), pp. 99-123, Croom Helm.

Table-1. Optimum dietary protein requirement (from various authors)

Organism	Source	Protein %
Penaeus setiferus	fish meal	28-32
P. japonicus	shrimp meal	40
"	casein	54
"	squid meal	60
"	casein & egg albumin	52-57
P. monodon	casein & fish meal	46
P. indicus	prawn meal	42.8
"	casein, arginine & cystine	39.0
P. merguiensis	Mytilus edulis meal	34-42
Cyprinus carpio	casein	31-38
Ictalurus punctatus	whole egg protein	32-36
Anguila japonica	casein, arginine & cystine	44.5
Ctenopharyngoden idella	casein	50
Fugu rubripes	casein	40-50
Epinephelus salmoides	Tuna muscle meal	40
Chanos chanos	casein	55
Chrysophrys major	casein	40
Tilapia aurea (fry)	casein & egg albumin	56
" (adult)	"	34
T. mosambica	white fish meal	40
T. zellii	casein	35
Microplerus dolomieri	casein & fish protein conc.	45
M. salmoides	"	40

Table-2. Requirement for essential amino acids in diet as percentage of protein and as percentage in diet ( ) - from various sources

	Anguilla japonica	Cyprinus carpio	Ictalurus punctatus	Chinook salmon	Generalised
Arginine	4.5 (1.7)	4.2 (1.6)	4.3 (1.03)	6.0 (2.4)	4.5
Histidine	2.1 (0.8)	2.1 (0.8)	1.5 (0.37)	1.8 (0.7)	1.7
Isoleucine	4.0 (1.5)	2.3 (0.9)	2.6 (0.62)	2.2 (0.9)	2.6
Leucine	5.3 (2.0)	3.4 (1.3)	3.5 (0.84)	3.9 (1.6)	4.0
Methionine (a)	5.0 (1.9)	3.1 (1.2)	2.3 (0.56)	4.0 (1.6) (b)	1.6(b) 2.5(a) 3.1(d)
Phenylalanine (c)	5.8 (2.2)	6.5 (2.5)	5.0 (1.20)	5.1 (2.1)	5.6(c)
Threonine	4.0 (1.5)	3.9 (1.5)	2.0 (0.53)	2.2 (0.9)	3.1
Tryptophan	1.1 (0.4)	0.8 (0.3)	0.5 (0.12)	0.5 (0.2)	0.6
Valine	4.0 (1.5)	3.6 (1.4)	3.0 (0.71)	3.2 (1.3)	3.2
Lysine	5.3 (2.0)	5.7 (2.2)	5.0 (1.50)	5.0 (2.0)	5.4
Protein in diet %	37.7	38.5	24.0	40.0	39

a - In the absence of cystine

b - In the presence of cystine

c - In the absence of tyrosine

d - In the presence of tyrosine

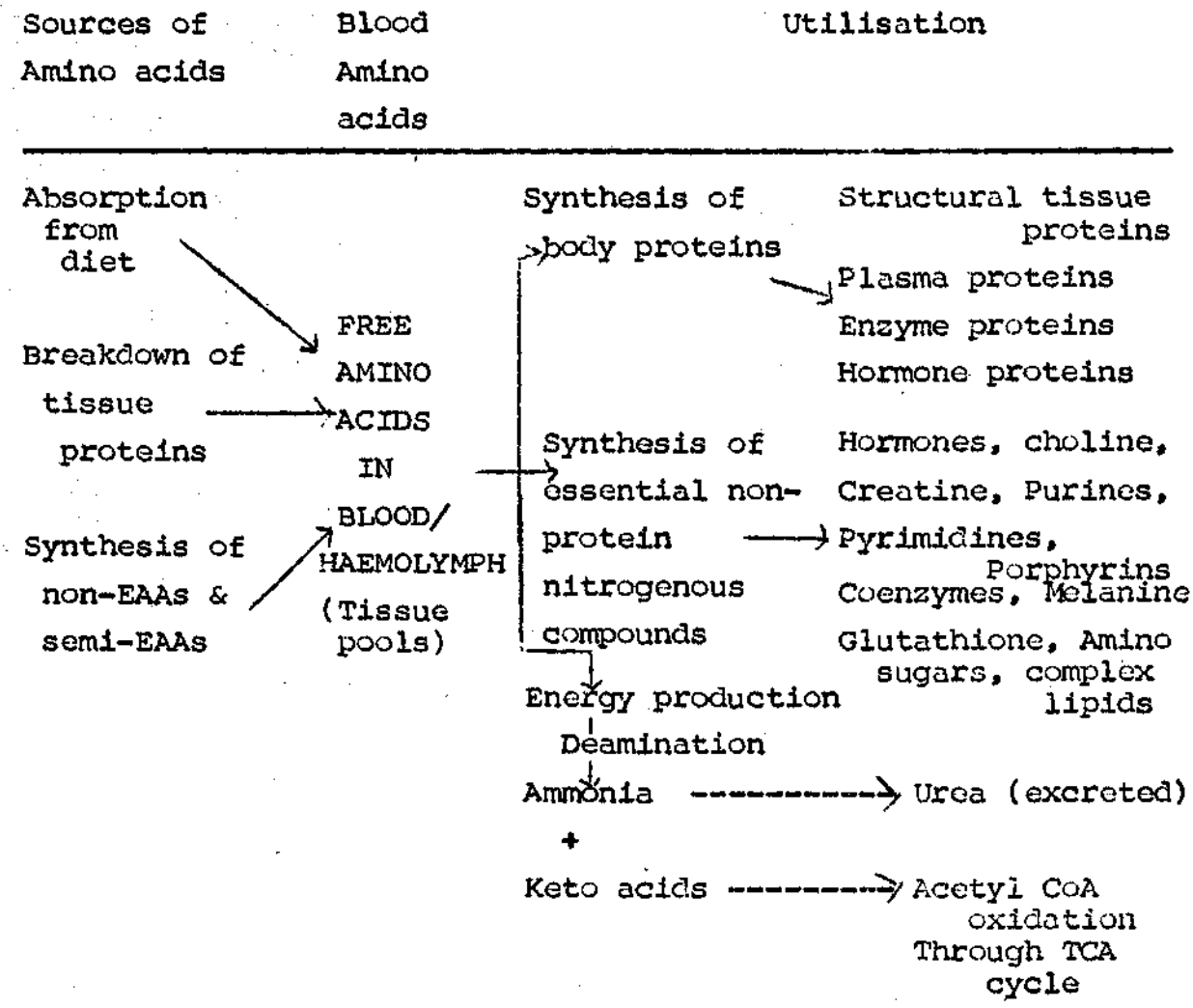

Figure 3. Diagram showing free amino acid pool as related to the metabolism of proteins.