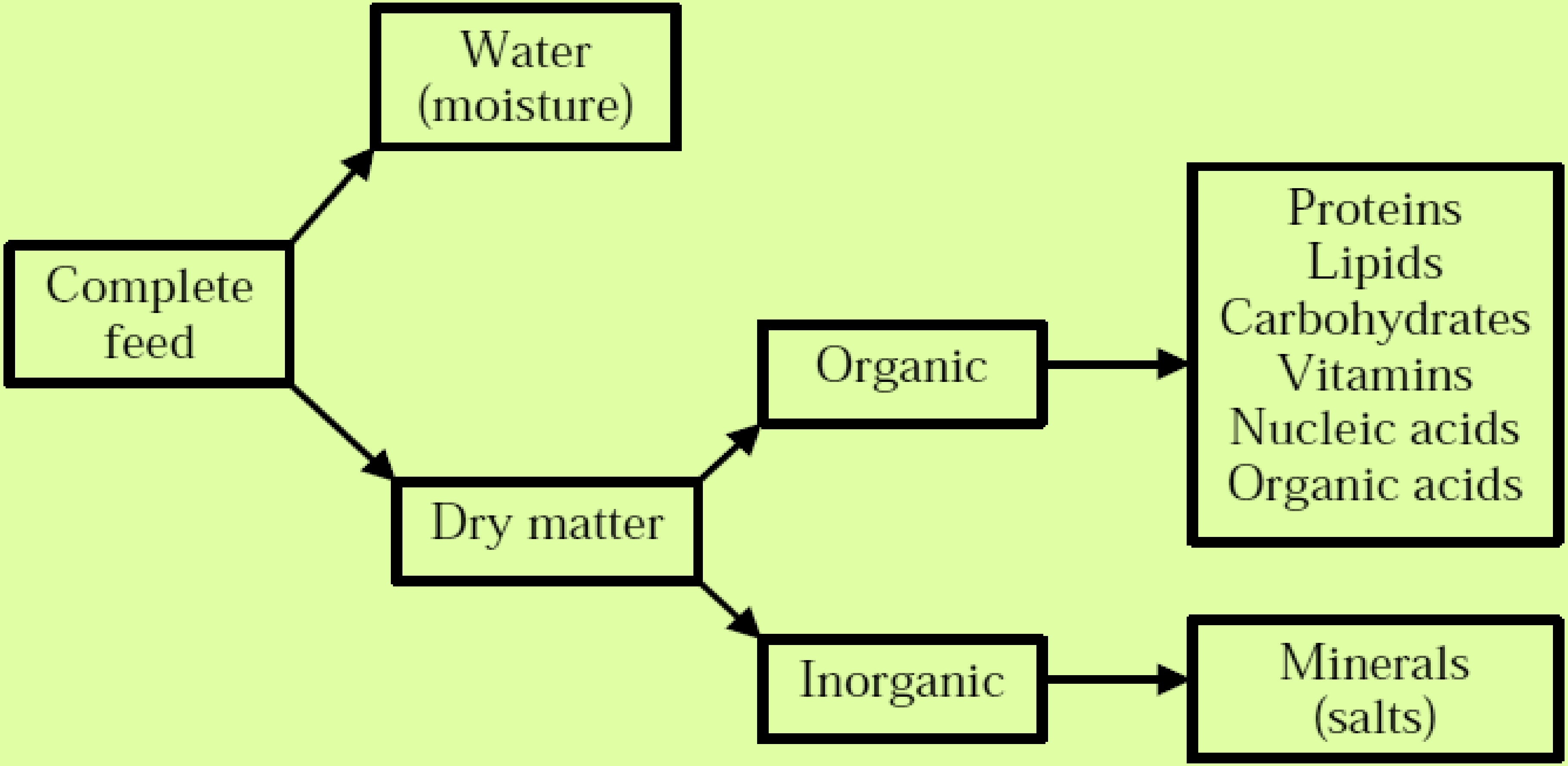

Fish Feeding:
3- Protein
Dr.A.Y.Al-Dubakel

Nutrient classes

Feeds and feedstuffs contain a range of chemical substances that can be grouped into classes according to constitution, properties and function

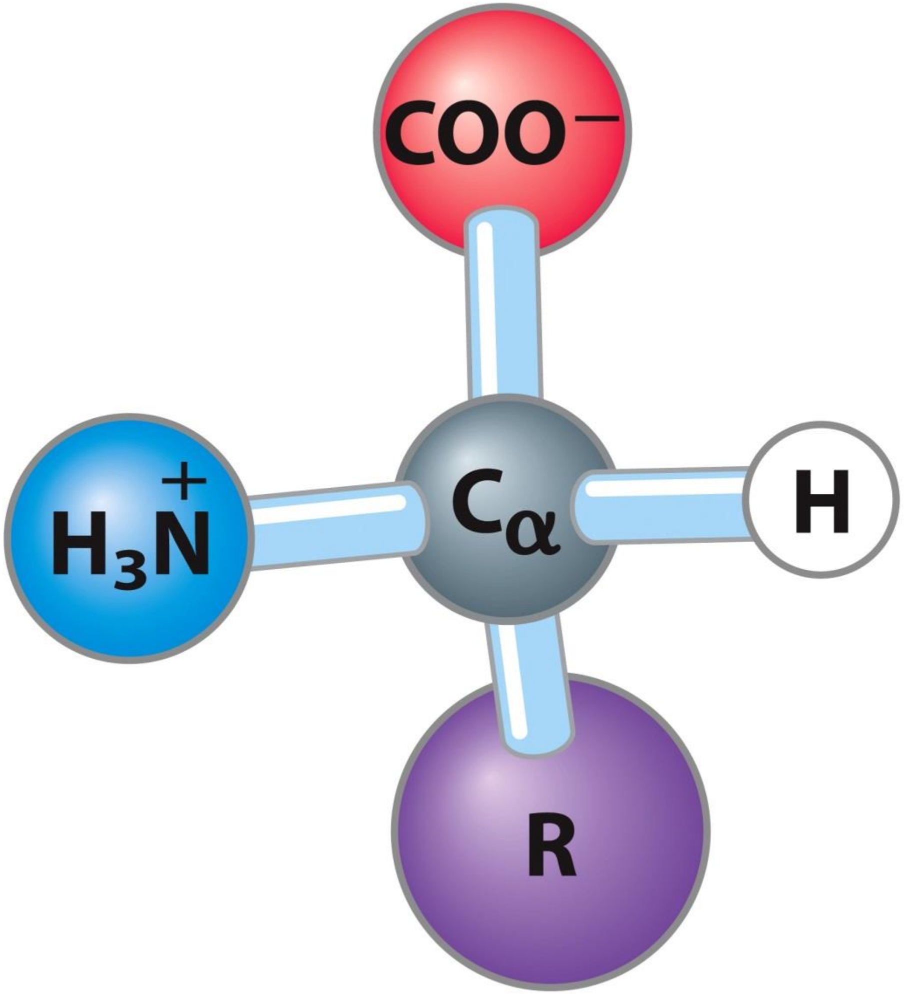


Hierarchical subdivision of a feed showing the major chemical components.

Proteins

Proteins are large organic, nitrogen-containing compounds comprising long chains of amino acids. The elemental compositions of proteins tend to be similar, with approximate percentages being C = 50–55%, H = 6–8%, O = 20–23%, N = 15–18% and S = 0–4%. The majority of the amino acids, of which 20 or so may be incorporated into proteins, have a chemical structure RCH(NH2)COOH, with a carboxyl (-COOH) and an amino (-NH2) group attached to the α carbon atom. R represents the side chain, which differs in configuration depending upon the amino acid. The amino acids can be divided into a number of series depending upon their structure and side chain configurations

Amino acids share many features, differing only at the R substituent



More than 700 amino acids occur naturally, but 20 (22)of them are especially important.

These 22 amino acids are the building blocks of proteins. All are α-amino acids.

Figure 3-2
Lehninger Principles of Biochemistry, Sixth Edition

Proteins

These essential and non-essential amino acids are listed in below

	Essential Amino Acids	Non-essential Amino Acids
1	Arginine	Cysteine
2	Histidine	Glutamine
3	Isoleucine	Glycine
4	Leucine	Proleine
5	Lysine	Serine
6	Methionine	Tyrosine
7	Phenylaline	Alanine
8	Threonine	Asparagine
9	Trytophan	Aspartic Acid
10	Valine	Glutamic Acid

20

21- Selenocysteine

22- Pyrrolysine

Chart 1. List of 21 Proteinogenic Amino Acids	
Amino Acid Name with (Abbreviation)	**Classification**
1. Histidine (His) 2. Isoleucine (Ile) 3. Leucine (Leu) 4. Lysine (Lys) 5. Methionine (Met) 6. Phenylalanine (Phe) 7. Threonine (Thr) 8. Tryptophan (Trp) 9. Valine (Val)	**ESSENTIAL Amino Acids** The 9 amino acids on the right are essential (vital), which means they are necessary for the human life and health but cannot be produced in your body so you need to get them from foods [1].
10. Arginine (Arg) 11. Cysteine (Cys) 12. Glutamine (Gln) 13. Glycine (Gly) 14. Proline (Pro) 15. Serine (Ser) 16. Tyrosine (Tyr)	**CONDITIONALLY ESSENTIAL Amino Acids** These amino acids can be synthesized in your body, but in certain circumstances, like young age, illness or hard exercise, you need to get them in additional amounts from foods to meet the body requirements for them. **Ornithine** is also considered conditionally essential amino acid, but it does not form proteins [2].
17. Alanine (Ala) 18. Asparagine (Asn) 19. Aspartic acid (Asp) 20. Glutamic acid (Glu) 21. Selenocysteine (Sec)	**NONESSENTIAL Amino Acids** These amino acids can be synthesized in your body from other amino acids, glucose and fatty acids, so you do not need to get them from foods [44].

Classified of Amino acids according to structure

(1) Aliphatic series – glycine, alanine, serine, threonine, valine, leucine, isoleucine.

(2) Aromatic series – phenylalanine, tyrosine.

(3) Sulphur amino acid series – cysteine, cystine, methionine.

(4) Heterocyclic series – tryptophan, proline, hydroxyproline.

(5) Acidic series – aspartic acid, glutamic acid.

(6) Basic series – arginine, histidine, lysine.

Proteins

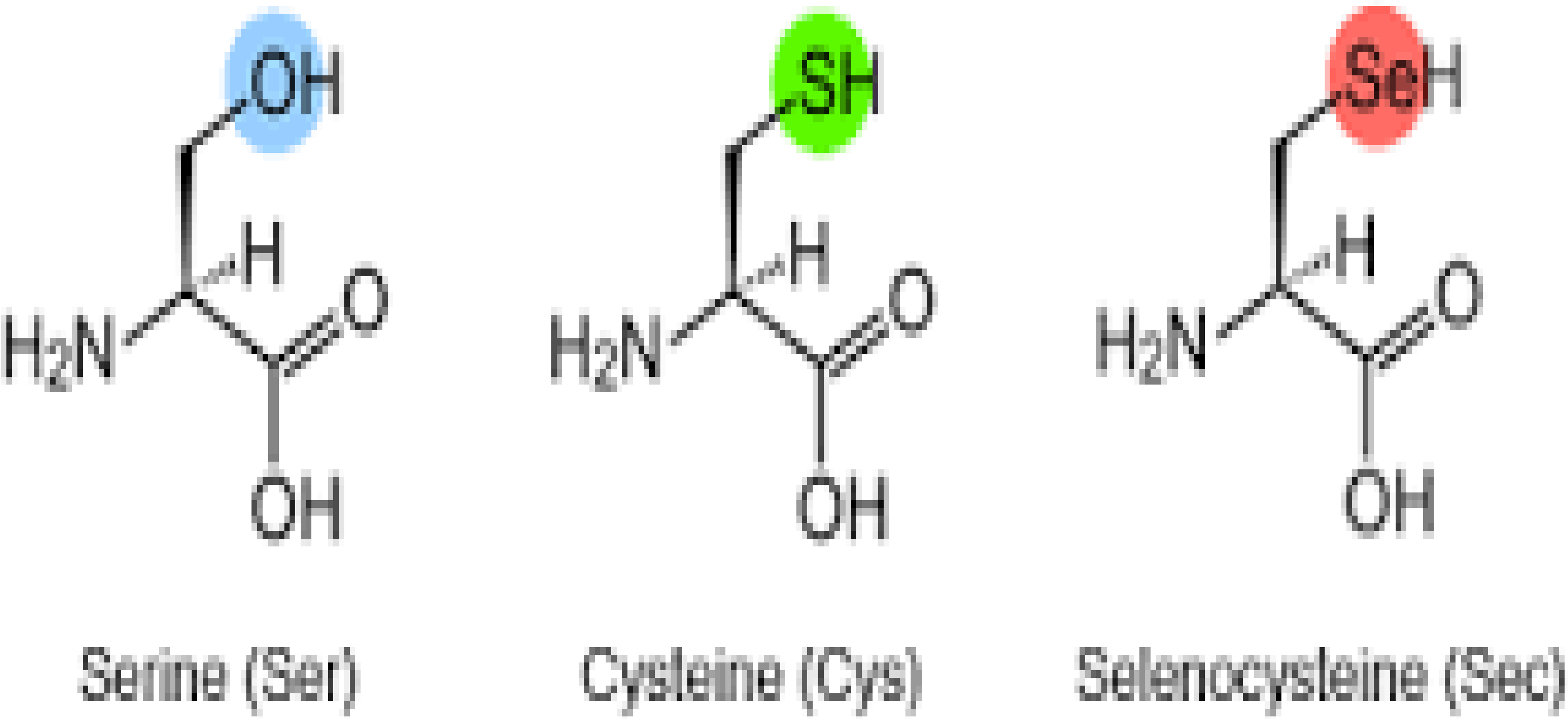
Table 4.Characteristics of the different series of amino acids. Indispensable (essential) amino acids are indicated in bold type, and conditionally indispensable amino acids are shown in italics.

Series	Characteristics	Amino acid
Aliphatic	Aliphatic amino acids containing one carboxyl group and one amino group	Gly, Ala, Ser, Val, **Thr**, **Leu**, **Ile**
Sulphur amino acids	Aliphatic, monoamino-monocarboxylic amino acids containing sulphur	**Met**, *Cys*
Acidic	Aliphatic, dicarboxylic amino acids; aqueous solutions are acidic	Asp, Glu
Basic	Aliphatic amino acids giving basic aqueous solutions	**Arg**, **His**, **Lys**
Aromatic	Amino acids with the aromatic, or benzenoid, ring	**Phe**, *Tyr*
Heterocyclic	Heterocyclic structure incorporating nitrogen; proline and hydroxyproline have an imino (NH) group, but no amino group	**Trp**, Pro, Hyp

21 amino acids form proteins. Selenocysteine as the 21st proteinogenic amino acid has been discovered only recently and this is why many health sites still mention only 20 amino acids.

Selenocysteine: An amino acid in which selenium takes the place of sulfur in the amino acid cysteine. Its formula is H-Se-CH2-CH(NH2)-COOH. Selenocysteine is an essential component of selenium-containing proteins, or selenoproteins.

It is found in tRNAs and in the catalytic site of some enzymes.



There are 22 amino acids, but only 20 amino acids are encoded by the universal genetic code.

Proteins "not included"

Two amide derivatives of amino acids are also found as constituents of proteins; these are:

1- The amides of aspartic

2- The amides of glutamic acids.

Asparagine, the β-amide of aspartic acid, is a constituent of many proteins, and it is hydrolysed to aspartic acid and NH3 by acid.

Glutamine, the γ-amide of glutamic acid, occurs in biological materials both in free form and as a constituent of proteins.

β-Alanine, an isomer of alanine, is not a constituent of proteins, but it occurs as the free amino acid, and as a component of several biological molecules: the vitamin pantothenic acid, coenzyme A, and the peptides carnosine and anserine found in muscle.

The two amino acids citrulline and ornithine are part of the metabolic sequence leading to the formation of urea; they are not constituents of proteins, but do occur in the free form in animal tissues.

Proteins "not included"

The structure of a protein is dependent upon the amino acid sequence (the primary structure) which determines the molecular conformation (secondary and tertiary structures). Proteins sometimes occur as molecular aggregates which are arranged in an orderly geometric fashion (quaternary structure). Constituents other than amino acids may be covalently bound and incorporated into proteins. For example, phosphoproteins such as milk casein and egg phosvitin contain phosphoric esters of serine, and glycoproteins such as collagen and some fish serum proteins contain monosaccharide or oligosaccharide units bound to serine, threonine or asparagine.

Proteins – or more correctly some of the amino acids they contain – are an essential component of the diet for all animals. The amino acids may, therefore, be classified according to whether or not protein synthesis and growth can proceed in the absence of a dietary supply .

***Essential amino acids* are those that animals cannot synthesis, or cannot synthesis in sufficient quantity to enable the maintenance of good rates of growth, whereas non-essential, or dispensable, amino acids can be synthesised *de novo* from other compounds.**

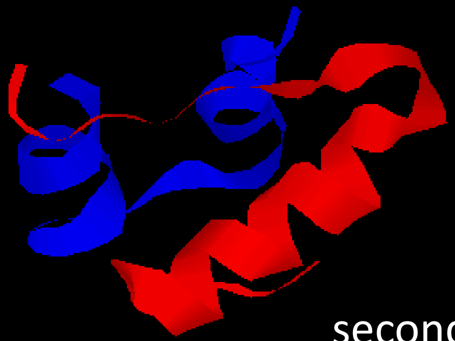
Proteins

- ***Essential or indispensable (10):*** *arginine, histidine, isoleucine, leucine, lysine, methionine,* **phenylalanine, threonine, tryptophan, valine.**
- ***Conditionally indispensable (2):*** *cystine, tyrosine.*
- ***Non-essential or dispensable (8):*** *alanine, asparagine, aspartic acid,serine glutamine, glutamic* **acid, glycine, proline.**

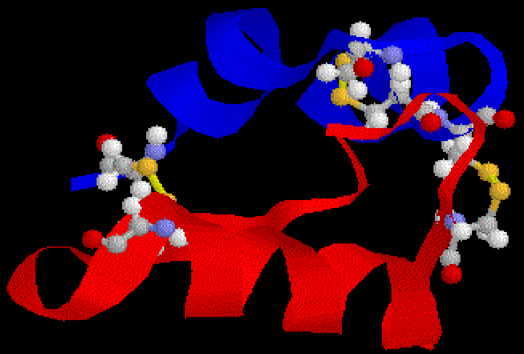
Some of the 10 essential amino acids can be replaced by their α-hydroxy or β-keto analogues, illustrating that it is the carbon skeleton of the essential amino acid that the animal is unable to synthesis. Cystine and tyrosine are classified as conditionally indispensable because they can be synthesised from being methionine and phenylalanine, respectively. Thus, a dietary supply of cystine and tyrosine is not required if sufficient quantities of their respective precursors are available. In addition to being components of proteins, several of the amino acids are precursors for biologically active molecules: for example, histidine is decarboxylated to form histamine, tyrosine is the precursor for thyroxine and catecholamines, and tryptophan is the precursor of serotonin (5-HT) and melatonin. Estimates of quantitative amino acid requirements have been made for a range of fish species, and the data have been summarised in several overviews.

Proteins

Essential idea: Proteins have a very wide range of functions in living organisms.



secondary



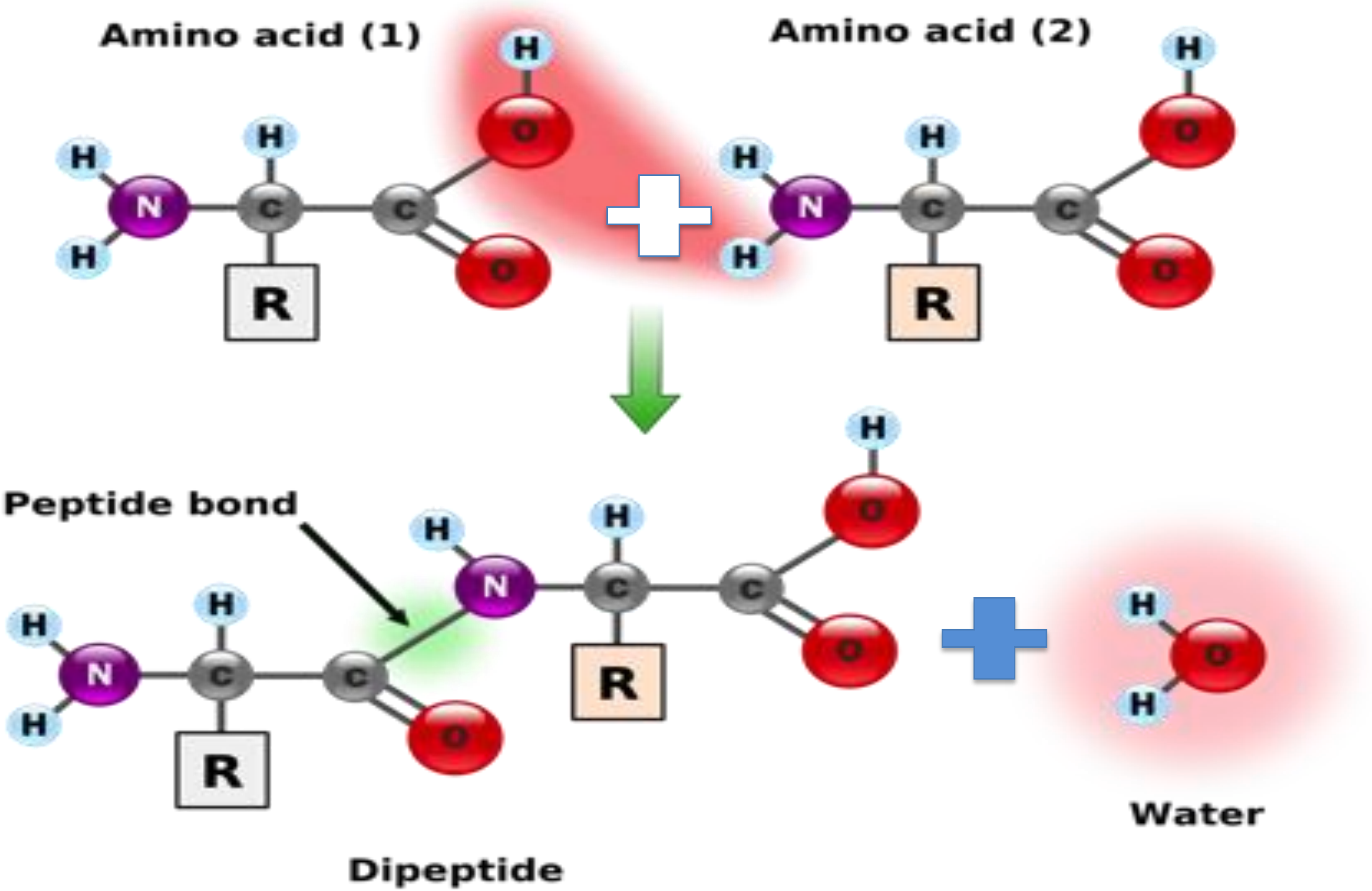
tertiary

quaternary

One of the central ideas in Biology is that structure dictates function. Above you can see insulin in its secondary, tertiary and quaternary structures. Polypeptides vary hugely in the combination and number of amino acids that they are composed from. Even if we consider a single polypeptide it's properties, and hence it's function, would vary greatly depending on it's level of structure. Insulin can exist in all these forms, but the active form, which controls blood glucose levels, is a the tertiary structure.

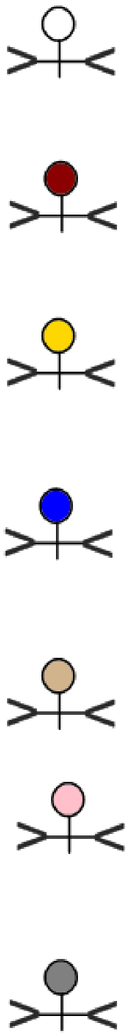
	Facts
1-	Amino acids are linked together to form polypeptides.
2-	There are 20 (22) different amino acids in polypeptides synthesized on ribosomes.
3-	Amino acids can be linked together in any sequence giving a huge range of possible polypeptides.
4-	The amino acid sequence of polypeptides is coded for by genes.
5-	A protein may consist of a single polypeptide or more than one polypeptide linked together.
6-	The amino acid sequence determines the three-dimensional conformation of a protein.
7-	Living organisms synthesize many different proteins with a wide range of functions.
8-	Every individual has a unique proteome. proteome is the entire set of proteins expressed by a genome
9-	Pepsin, insulin, rhodopsin (protein involved in visual), collagen and spider silk as examples of the range of protein functions.
10-	Denaturation of proteins by heat or by deviation of pH from the optimum.

A ribosome **condenses** two amino acids into a dipeptide forming a peptide bond

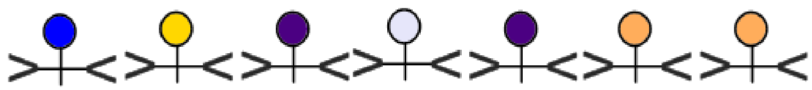


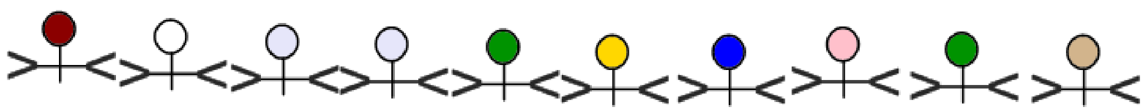
Why are there infinite possibilities of polypeptides?

- could be any length
- 20 amino acids
- amino acids in any order or combination

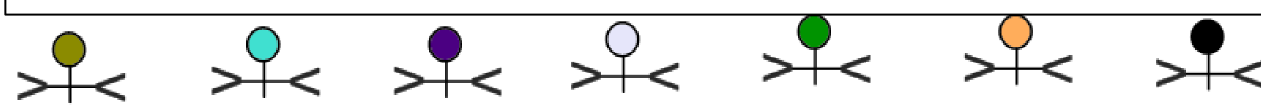


If a polypeptide contains just 7 amino acids there can be 20^7 = 1,280,000,000 possible polypeptides generated.





Given that polypeptides can contain up to 30,000 amino acids (e.g. Titin elasticity of muscle) the different possible combinations of polypeptides are effectively infinite.



Proteins Classification

1- Classification based on the source of protein moleucle.

2- Classification based on the shape of protein molecule.

3- Classification based on composition and solubility.

4- Classification based on biological function

1-The source of protein moleucle.

Animal proteins are the proteins derived from animal sources such as eggs, milk, meat and fish. They are usually called *higher-quality proteins* because they contain (and hence supply) adequate amounts of all the essential amino acids.

Plant proteins are called *lower-quality proteins* since they have a low content (limiting amount) of one or more of the essential amino acids.

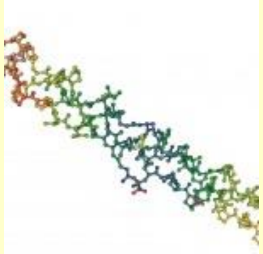
The four most common limiting amino acids are methionine, lysine, threonine and tryptophan

Limiting amino acids in some plant proteins

Food	*Amino acid(s)*
Cereal grains and millets	Lysine, Threonine
Rice and soybeans	Methionine
Legumes (peas and beans)	Methionine, Tryptophan
Groundnuts	Methionine, Lysine, Threonine
Sunflower seeds	Lysine
Green leafy vegetables	Methionine

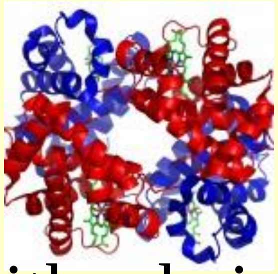
2- THE SHAPE OF PROTEIN MOLECULE.

1- Fibrous protein



Collagens — connective tissue
Elastin — arteries
Keratin — hair & wool

2- Globular (Corpuscular) proteins



albumins
Globulins
histones

- Proteins are commonly described as either being fibrous or globular in nature.
- Fibrous proteins have structural roles whereas globular proteins are functional (active in a cell's metabolism).

In globular proteins the hydrophobic R groups are folded into the core of the molecule, away from the surrounding water molecules, this makes them soluble. In fibrous proteins the hydrophobic R groups are exposed and therefore the molecule is insoluble.

Properties	**Fibrous Protein**	**Globular Protein**
Shape	Long and narrow	Rounded / spherical
Role	Structural (strength and support)	Functional (catalytic, transport, etc.)
Solubility	(Generally) insoluble in water	(Generally) soluble in water
Sequence	Repetitive amino acid sequence	Irregular amino acid sequence
Stability	Less sensitive to changes in heat, pH, etc.	More sensitive to changes in heat, pH, etc.
Examples	Collagen, myosin, fibrin, actin, keratin, elastin	Catalase, haemoglobin, insulin, immunoglobulin

3- COMPOSITION AND SOLUBILITY.

1- Simple Proteins or Holoproteins :proteins containing only amino acids

2- Conjugated or Complex Proteins or Heteroproteins: proteins linked with a separable nonprotein portion

Phosphoproteins - Glycoproteins-lipoproteins

chromoproteins - nucleoproteins

4- BIOLOGICAL FUNCTION

Class of protein	*Function*	*Examples*
Enzymic proteins	Biological catalysts	Urease, Amylase, Catalase, Cytochrome C, Alcohol dehydrogenase.
Structural proteins	Strengthening or protecting biological structures	Collagen, Elastin, Keratin, Fibroin
Transport or carrier proteins	Transport of ions or molecules in the body	Myoglobin, Hemoglobin, Ceruloplasmin, Lipoproteins
Nutrient and storage proteins	Provide nutrition to growing embryos and store ions	Ovalbumin, Casein, Ferritin
Contractile or motile proteins	Function in the contractile system	Actin, Myosin, Tubulin
Defense proteins	Defend against other organisms	Antibodies, Fibrinogen, Thrombin
Regulatory proteins	Regulate cellular or metabolic activities	Insulin, G proteins, Growth hormone
Toxic proteins	Hydrolyze (or degrade) enzymes	Snake venom, Ricin.

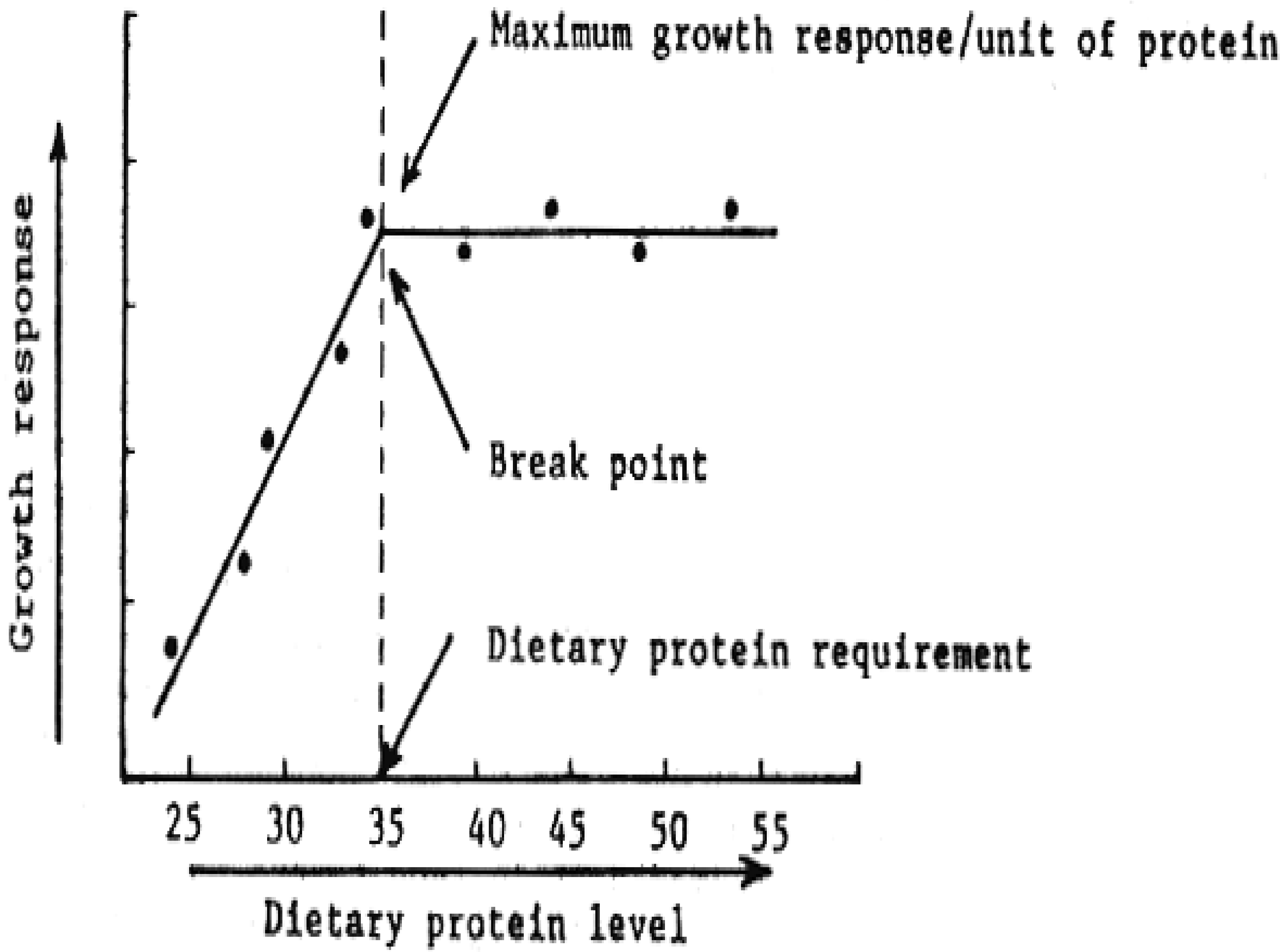
Functions of Proteins

- Protein is vital in the maintenance of body tissue, including development and repair.
- Protein is the major source of energy.
- Protein is involved in the creation of some hormones, help control body functions that involve the interaction of several organs and help regulate cell growth.
- Protein produces enzymes that increase the rate of chemical reactions in the body.

Protein requirements Experiments

Optimum dietary protein level

Based on feeding techniques developed for terrestrial animals the dietary protein requirements of fish were first investigated in the Chinnok salmon (*Oncorhynchus tshawytscha*) by Delong *et al.*, (1958). Fish were fed a balanced diet containing graded levels of a high quality protein (casein:gelatin mixture supplemented with crystalline amino acids to simulate the amino acid profile of whole hen's egg protein) over a 10-week period and the observed protein level giving optimum growth was taken as the requirement .Since these early studies the approach used by workers today has changed very little if at all, with the possible exception of the use by some researchers of maximum tissue protein retention or nitrogen balance in preference to weight gain as the criterion of requirement (Ogino, 1980). Dietary protein requirements are normally expressed in terms of a fixed dietary percentage or as a ratio of protein to dietary energy.



Maximum growth response/unit of protein
Break point
Dietary protein requirement
Growth response
25
30
35
40
45
50
55
Dietary protein level

Evaluation of Diet Proteins

<u>Protein efficiency ratio (PER)</u> Defined as the grams of weight gained per gram of protein consumed.

$$PER = \frac{\text{Weight gain}^*}{\text{Protein consumed}}$$

*** With this method no allowance is made for maintenance: ie. method assumes that all protein is used for growth.**

<u>Apparent net protein utilization (Apparent NPU)</u> Defined as the percentage of ingested protein which is deposited as tissue protein.

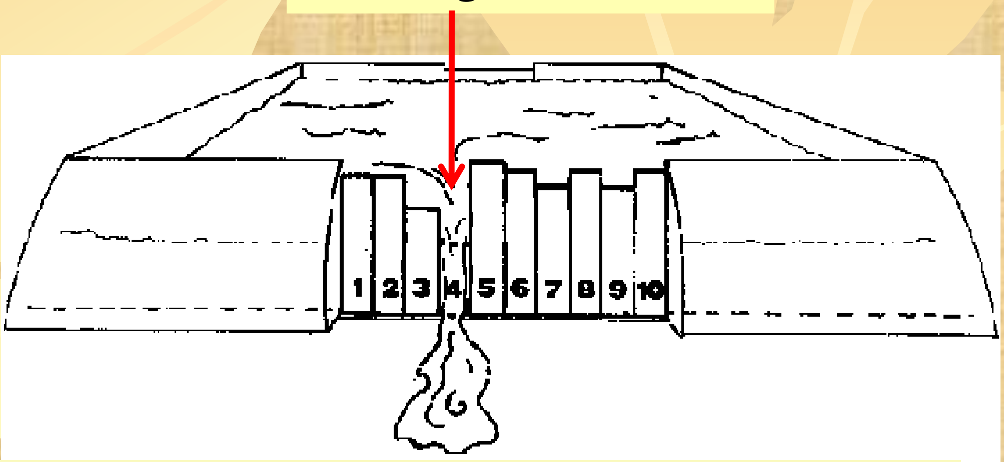
$$\text{Apparent NPU} = \frac{Pb - Pa}{Pi} \times 100$$

where Pb is the total body protein at the end of the feeding trial, Pa is the total body protein at the beginning of the feeding trial, and Pi is the amount of protein consumed over the feeding trial

Proteins

Limiting Amino Acids

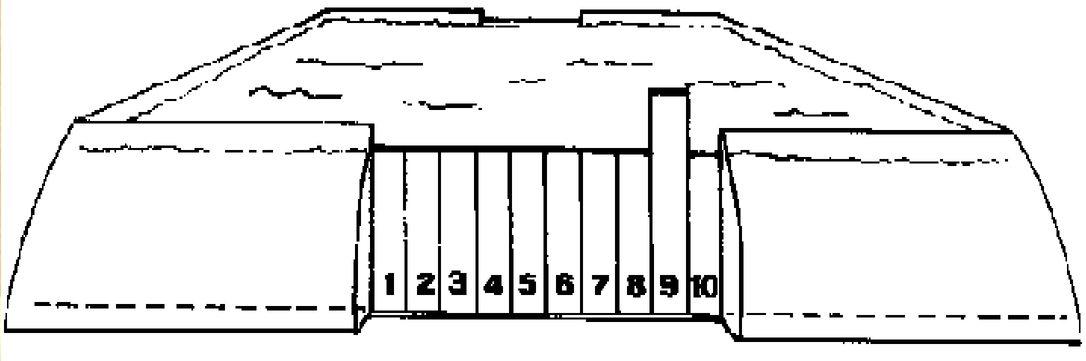
Limiting Amino Acids



the level of water in the pond will depend on the height of the shortest plank (plank 4). The shortest plank represents the 'first limiting amino acid'.

If this plank is lengthened (or this amino acid is increased in level by altering the proportion or the type of ingredients used, or by adding it in synthetic form) then plank 3 would control the water level (or be the next limiting amino acid). Ideally all the planks should be just as high as the level of water desired in the pond (the quantity of each amino acid in the diet at exactly the correct level for the species being cultured) to avoid wastage of materials.

what happens if one plank is unnecessarily long (or if one amino acid is present in excess in the diet) - it serves no useful purpose and is an unnecessary expense.



Proteins

Limiting Amino Acids and Chemical Score of Essential Amino Acid Content of Selected Feed Proteins

Scores based on comparison with whole egg protein of the following amino acid composition (percentage of protein): arginine, 6.7; cystine, 2.2; histidine, 2.7; isoleucine, 7.0; leucine, 8.5; lysine, 6.8; methionine, 3.3; phenylalanine, 5.4; threonine, 5.5; tryptophan, 1.9; and valine, 8.2.

Feedstuff	Arg	His	Iso	Leu	Lys	Met + Cys	Phe	Thr	Try	Val
Fish meal	85	85	66	88	110	71	78	74	58	61
Meat meal	77	96	28*	100	86	36*	72	60	68	75
Milk, skimmed	53*	92	88	110	104	69	91	80	73	75
Milk, whole	60	100	100	136	106	83	92	83	84	78
Soybean meal	110	89	66	92	90	54*	102	69	68	63
Cottonseed cake	164	96	46*	69	60	51*	100	58	58	55
Sunflower oil cake	112	59	46*	62	32*	22*	61	47*	79	46*
Red bean	112	130	77	49*	128	27*	107	83	42*	72
Chlorella	77	55	64	90	44*	47*	92	100	68	72
Spirulina	97	66	86	94	67	33*	92	83	73	79
yeast	77	81	68	94	111	51*	81	91	63	66
Brewer's spent grains	68	66	77	97	48*	22*	87	58	68	66

Proteins

.Dietary protein requirement of fish and shrimp (expressed as % of dry diet)

Species	Dietary protein requirement	Size range	Feeding regime	Culture system
Cyprinus carpio	35	Grower	5%bw/d	Indoor/tank
C. carpio	38	Fingerling	*Ad lib*.	Indoor/tank
Ctenopharyngodon idella	41–43	Fry	Fixed	Indoor/tank
Mugil capito	24	Fingerling	*Ad lib*.	Indoor/tank
Ictalurus punctatus	35	Grower	Fixed (1–4%bw/d)	Outdoor/cage
M. saxatilis	55	Fingerling	*Ad lib*.	Indoor/tank
Pleuronectes platessa	50	Juvenile	*Ad lib*.	Indoor/tank
Salmo gairdneri	40–45	Fingerling/juv.	*Ad lib*.	Indoor/tank
Penaeus setiferus	28–32	Juveniles 4g	5%bw/d	Indoor/tank

ad libitum

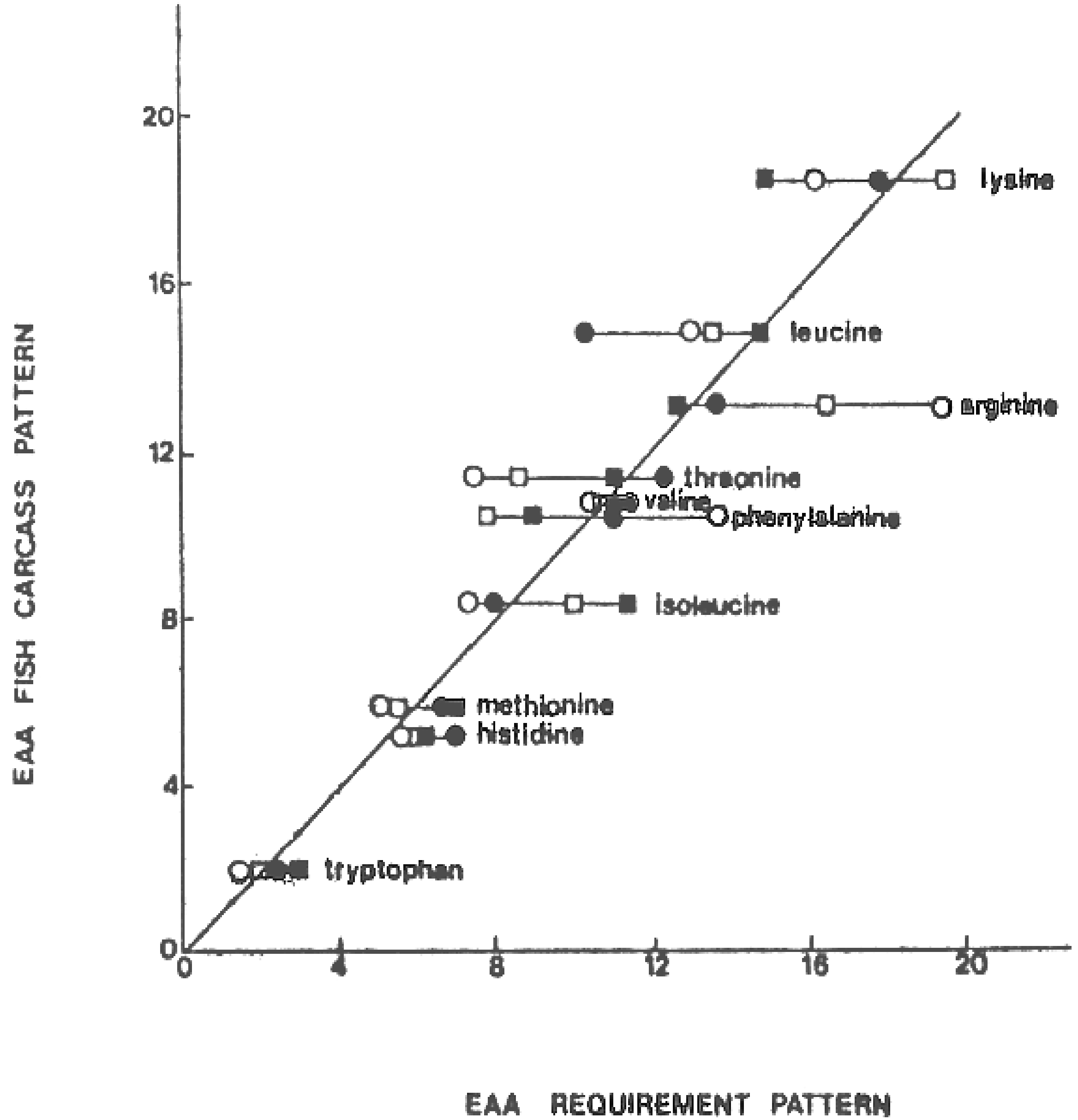
Proteins

Quantitative dietary EAA requirement of fish as % of protein and % diet (EAA /p)

Species	Histidine	Isoleucine	Leucine	Lysine	Methionine	Arginine
C. carpio	2.1 (0.8/39)	2.5 (0.9/38)	3.3 (1.3/38)	5.7 (2.2/38)	2.1 (0.8/38)	3.3 (1.3/38)
I. punctatus	1.5 (0.4/24)	2.5 (0.6/24)	3.5 (0.8/24)	5.1 (1.5/30)	1.34 (0.3/24)	4.3 (1.03/24)
O. tshawytscha	1.8 (0.7/40)	2.2 (0.9/41)	3.9 (1.6/41)	5.0 (2.0/40)	1.5 (0.6/40)	6.0 (2.4/40)
S. gairdneri	-	-	-	3.7 (1.3/35)	-	>4.0 (1.4/35)
S. gairdneri	-	-	-	6.1 (2.9/47)	-	5.9 (2.5/47)
S. gairdneri	-	-	-	-	1.57 (0.5/35)	-
O. mossambicus	-	-	-	4.1 (1.6/40)	<1.33 (0.5/40)	<4.0 (1.5/40)

Proteins

The relation between EAA requirement patterns and and patterns of the same EAA in the fish carcass of four spp.
carp (•), Japanese eel (■), channel catfish (□) and chinook salmon (o)



Proteins

Dietary Essential Amino Acid Deficiency

Limiting EAA	Fish	Deficiency signs
Lysine	*Salmo gairdneri*	Dorsal/caudal fin erosions , increased mortality
	Cyprinus carpio	Increased mortality
Methionine	*S. gairdneri*	Cataract
	Salmo salar	Cataract
Tryptophan	*S. gairdneri*	Scoliosis; cataract ; caudal fin erosion; decreased carcass lipid content , elevated Ca, Mg, Na and K carcass concentration .
	Oncorhynchus nerka	Scoliosis .
Miscellaneous	*O. keta*	Scoliosis
	C. carpio	Increased mortality and incidence of lordosis observed with dietary defi- ciencies of leucine, isoleucine, lysine, arginine and histidine .

Proteins

Non-protein nitrogenous constituents

Amino acids are important not only as building blocks of protein but as the primary constituents or nitrogen precursors for many non-protein nitrogen containing compounds. Some of the more biologically important non-protein nitrogenous compounds that originate from amino acids.

Nitrogenous compound	Amino acid precursor	Physiological function of compound
Purines & pyrimidines	Glycine & aspartic acid	Constituents of nucleotides and nucleic acids
Creatine	Glycine & arginine	Energy storage as creatine phosphate in muscle
Bile acids (glycoholic & taurocholic acids)	Glycine & cysteine	Bile acids, aid in fat digestion and absorption
Thyroxine, epinephrine & norepinephrine	Tyrosine	Hormones
Ethanolamine & choline	Serine	Constituents of phospholipids
Histamine	Histidine	A vasodepressor
Serotonin	Tryptophan	Transmission of nerve impulses
Porphyrins	Glycine	Constituents of haemoglobin and cytochromes
Niacin	Tryptophan	Vitamin
Melanin	Tyrosine	Pigment of skin and eyes