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EFFECT OF DELTAMETHRIN ON *CIPRINUS CARPIO* WITH SPECIAL REFERENCE TO PROTEIN AND AMINO ACID METABOLISM

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ABSTRACT

Present study is aimed at to investigate the effect of two different concentrations of (5ppm, 10ppm) deltamethrin in *Ciprinus carpio* different tissues i.e Liver, Heart, Kidney and Brain with reference to Protein and amino acid metabolism. The fishes were randomly divided into 3 groups having 6 in each group: (1) Control (2) Deltamethrin-experimental toxic group (5ppm) (3) Deltamethrin- experimental toxic group (10 ppm). Deltamethrin- experimental groups decreased the contents of total proteins (TP), soluble proteins (SP), structural proteins (STP) and increased the levels of free amino acid (FAA), aspartate aminotransferase (AAT), alanine aminotransferase (ALAT), lucine aminotransferase (LAT), isoleucine aminotransferase (ILAT), valine aminotransferase (VAT) in all the fish organs (Liver, Heart, Kidney and Brain). From the results it is assumed that the Deltamethrin causes potential damage protein and amino acid metabolism in different organs of fish.

Key words: Deltamethrin, *Ciprinus carpio*, Protein, Amino acid.

INTRODUCTION

Proteins perform multiple critically important roles. An internal protein network, the cytoskeleton, maintains cellular shape and physical integrity. Actin and myosin filaments form the contractile machinery of muscle. Hemoglobin transports oxygen, while circulating antibodies search out foreign invaders. Enzymes catalyze reactions that generate energy, synthesize and degrade bio-molecules, replicate and transcribe genes, process mRNAs, etc. Receptors enable cells to sense and respond to hormones and other environmental cues. An important goal of molecular medicine is the identification of proteins whose presence, absence, or deficiency is associated with specific physiologic states or diseases [1]. These are hydrolyzed to amino acids in the body which are further metabolized by incorporation into proteins or deamination or oxidation of amino acids [1]. Although body proteins represent a significant proportion of potential energy reserves, under normal circumstances they are not used for energy production. In an extended fast, however, muscle protein is degraded to amino acids for the synthesis of essential proteins and for gluconeogenesis to maintain blood glucose concentration. This accounts for the loss of muscle mass during fasting. Efficient metabolism is controlled by orderly, sequential, and branching metabolic pathways.

Protein budget of a cell can be taken as an important diagnostic tool in the evaluation of its physiological standards [2]. Soluble proteins represent enzymes, hormones and various polypeptides, which play a dynamic and key role in various physiological activities. The cellular proteins exist in soluble and insoluble form and the level of soluble protein is generally considered to be an index of aqueous state of cytosol which corresponds to the active metabolic state. In the protein molecules the α – amino group is removed and the resulting skeleton is converted in to major metabolic intermediate. Hydrolysis of dietary protein and endogenous protein results in the formation of amino acid pool in the body. The

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physiological state of the cell depends upon its free amino acid pool. The protein constituents of plasma include a number of enzymes, some of which have clinical diagnostic importance Ex: amylase, lipase, phosphatases, aminotransferases and glycolytic enzymes. When amino acids are metabolized, the resulting excess nitrogen must be excreted. As the primary form in which the nitrogen is removed from amino acids is ammonia, and because free ammonia is quite toxic, humans and higher animals rapidly convert the ammonia derived from amino acid catabolism to urea, which is neutral, less toxic, very soluble, and excreted in the urine. Thus the primary nitrogenous excretion product in humans is urea, produced by urea cycle in liver. The carbon skeletons of many amino acids may be derived from metabolites in central pathways, allowing the biosynthesis of some, but not all. Amino acids that can be synthesized in this way are therefore not required in the diet (non-essential amino acids), whereas amino acids having carbon skeletons that cannot be derived from normal human metabolism must be supplied in the diet (essential amino acids). In contrast to the catabolic process, for the biosynthesis of non-essential amino acids, amino groups must be added to the appropriate carbon skeletons. This generally occurs through the transamination of an α-keto acid corresponding to that specific amino acid [3].

Amino acids normally serve as substrates for the synthesis of the body's own proteins, rather than as source of energy. However, during a prolonged fast, or after illness or injury, proteins are degraded and the constituent amino acids are converted into glucose [3]. Amino acids may not only act as precursors for the synthesis of essential proteins, but also contribute towards gluconeogenesis, glycogenesis and keto acid synthesis[1]. Biological value of proteins is considered on the basis of tissue amino acid composition. The free amino acid content acts as precursors for protein synthesis and gluconeogenesis in all most all tissues [1]. In protein the amino acids are aggregated into long –chains by means of peptide linkage [4]. The amino acids released during protein degradation due to activation of proteolysis will once again return to the amino acid pool and thus the free amino acids are the currency through which protein metabolism operates, showing the interdependence of both amino acids and proteins. Hydrolysis of proteins is quite common phenomenon wherein proteases split proteins stepwise into amino acids. Liver is exclusive site for the metabolism of several amino acids and the free amino acid content of liver is known to change during physiological and pathological conditions. The principal mechanism for removal of amino groups from the common amino acids is via transamination, or the transferring the amino group from the amino acid to a suitable α-keto acid acceptor, most commonly to α-ketoglutarate or oxaloacetate. Several enzymes, called aminotransferases (or transaminases), are capable of removing the amino group from most amino acids and producing the corresponding α-keto acid. Aminotransferase enzymes use pridoxal phosphate, a cofactor derived from the vitamin B6 pyridoxine, as a key component is their catalytic mechanism [3]. Transaminases are important enzymes in animal metabolism which are intimately associated with amino acid synthesis and lysis. Among these, aspartate and alanine transaminases (AAT and ALAT) are widely distributed in the cells of all animals. The AAT catalyses the interconversion of aspartic acid and α-ketoglutaric acid to oxaloacetic acid and glutamic acid. While ALAT catalyses the interconversion of alanine and α-ketoglutaric acid to pyruvic acid and glutamic acid. Branched chain amino acids (BCAAs) leucine, isoleucine and valine are essential amino acids and the precise regulation of these amino acids depends on endogenous proteolysis [5]. The metabolism of BCAAs is intiated by respective branched chain aminotransferases (BCAT) resulting in glutamate and corresponding branched chain keto acids [6]. These transaminases also enzymes function as a link between protein and carbohydrate metabolisms and the net outcome is incorporation of keto acids into the TCA cycle; besides these enzymes are the first in their catabolic pathways and thus limit the overall reaction rates. There is much evidence for the alteration in the activities of these enzymes to a variety of environmental and physiological conditions [7]. Deltamethrin, a synthetic pyrethroid pesticide contaminating aquatic ecosystems as a pollutant, Pesticides applied to the land may be washed into surface waters and may kill or at least adversely influence the life of aquatic organisms [8].

In view of the importance of protein and amino acid metabolisms, the present study is taken up to study the alterations in protein profiles, free amino acid composition and their turnover in different organs of fish during deltamethrin toxic condition.

MATERIALS AND METHODS

Experimental animals

The tests were performed in a concrete holding tanks, glass aquaria, constant supply of water and good lighting system. The indoor tanks were filled with tap water and aerated for 3 days to help reduce the chlorine content. About 300 active test specimens ranging between 5 and 10 cm standard length were transported to the laboratory from a farm. The specimens were acclimatized to laboratory conditions for 7 days in the indoor holding tanks. The pH, dissolved oxygen concentration and temperature of water in the tanks were monitored.

Preliminary tests were conducted to provide guidance on range of concentration of pesticide to use in the bioassay. A stock solution of 25 mg/l was prepared from the original product concentration of 12.5 g/l. From the stock solution, the test solutions were prepared using distilled water. The specimens were not fed a day prior to and during toxicity tests to reduce faecal and excess food

contaminating the test solution. The nominal test concentrations were 5ppm&10ppm with six replicates each. The results from the toxicity tests were analyzed, using a World Health Organisation (WHO) Computer Programme, Probit (1982). The concentrations used were converted by the programme to log dose and the number of dead fishes to mortality Probit values. A plot of these two parameters was made from which the LC50 was estimated.

The fishes were maintained according to the ethical guidelines for animal protection and welfare bearing the CPCSEA 438/01/a/cpcsea/dt 19.05.2007 in its resolution No: 9/IAEC/SVU/SK/2012/dt 11.05.2012.

Selection of Pesticide

Deltamethrin, a synthetic pyrethroid pesticide, was selected for the present study. It was obtained as commercial grade chemical from Sigma chemicals, USA.

Experimental design

The fishes were divided into 3 groups, each consisted of 6 and used for studying the effects of different concentrations of deltamethrin.

Group 1 - Control
Group 2 - 5ppm concentration
Group 3 - 10ppm concentration

Isolation of Tissues

The animals were sacrificed. Functionally different organs such as Liver, Heart, Kidney and Brain were separated and frozen in liquid nitrogen (-180^{0}C) and stored at -40^{0}C until further use. At the time of analyses the tissues were thawed and selected parameters were estimated by employing standard methods.

Procurement of Chemicals

All chemicals used in the present study were Analar grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fisher (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

Biochemical Analyses

The protein content was estimated by the method of Lowry *et al*. (1951) [9]. The total free amino acid content was estimated by the method of Moore and Stein (1954) [10]. Aspartate aminotransferase activity was assayed by the method of Reitman and Frankel (1957) [11]. Alanine aminotransferase activity was assayed by the method of Reitman and Frankel (1957) [11]. Branched chain aminotransferases (LAT, ILAT and VAT) were estimated by the method of Taylor and Jenkins (1966) [12].

Statistical treatment of data

All assays were carried out with six separate replicates from each group. The mean, standard error (SE) and Analysis of Variance (ANOVA) were done using SPSS statistical software for different parameters. Difference between control and experimental assays was considered as significant at $P<0.05$.

RESULTS

Total, soluble and structural proteins were decreased in all the tissues of fish when compared control with experimental groups (Tables 1-4). An increase in the levels of Free amino acids (FAA), Aspartate (AAT), Alanine (ALAT), Leucine (LAT), Isoleucine (ILAT), and Valine (VAT) aminotransferase activity levels were elevated in all the tissues of fish when compared to control and were increased throughout dealing with different concentrations of deltamethrin (Tables 1-4).

DISCUSSION

In the present investigation the total and soluble proteins were decreased significantly in all the tissues during deltamethrin toxic condition. Consistent with the decreased protein content, the free amino acid levels were elevated in all the tissues during deltamethrin toxic condition. The decreased protein and increased free amino acid content may be correlated to the augmented proteolysis coupled with reduced protein synthesis during experiment with deltamethrin. It has been well established that maintenance of structural integrity in a highly organized state requires a continuous supply of energy. The impairment of energy supply ultimately leads to denaturation of protein through the activation of proteolytic enzymes [13]. Hence, the decreased protein content with subsequent elevation in FAA content may be correlated to the augmented proteolysis coupled with decreased protein synthesis during deltamethrin toxic condition. The elevated FAA levels also indicate altered nitrogen homeostasis. (Figure 1)

Aminotransferase (transaminase) reactions form pyruvate from alanine, oxaloacetate from aspartate, and α-ketoglutarate from glutamate. Because these reactions are reversible, the cycle also serves as a source of carbon skeletons for the synthesis of these amino acids. Other amino acids contribute to gluconeogenesis because their carbon skeletons give rise to citric acid cycle intermediates. Alanine, cysteine, glycine, hydroxyproline, serine, threonine, and tryptophan yield pyruvate; arginine, histidine, glutamine, and proline yield α-ketoglutarate; isoleucine, methionine, and valine yield succinyl-CoA; and tyrosine and phenylalanine yield fumarate.(Figure 2).

The citric acid cycle is the final pathway for the oxidation of carbohydrate, lipid, and protein whose common end-metabolite, acetyl-CoA, reacts with oxaloacetate to form citrate. By a series of dehydrogenations and decarboxylations, citrate is degraded, releasing reduced coenzymes and $2CO_2$ and regenerating oxaloacetate. The reduced coenzymes are oxidized by the respiratory chain linked to formation of ATP. Thus, the cycle is the major route for the generation

of ATP and is located in the matrix of mitochondria adjacent to the enzymes of the respiratory chain and oxidative phosphorylation. The citric acid cycle is amphibolic, since in addition to oxidation it is important in the provision of carbon skeletons for gluconeogenesis, fatty acid synthesis, and interconversion of amino acids.

All the transamination reactions (AAT, ALAT and BCATs) were significantly elevated in all the tissues during deltamethrin toxic condition indicating an increased amino acid turnover. The elevated transaminase reactions as observed in the present study agree with the above findings of elevated levels of excitatory amino acid, glutamate in all the tissues. The elevated transaminase activities as observed in the present study coupled with elevated levels of excitatory amino acid neurotransmitter, glutamate, suggest the possible contribution of these metabolic pathways in the endogenous production of glutamate thus causing deltamethrin toxic state.

The concentration of proteins in a tissue is generally determined by the balance between rate of synthesis and degradation or catabolism [13] and the overall nitrogen homeostasis in animal is the outcome of integrated metabolic functions of the tissues [14]. The degradation of proteins was mainly brought about by protein hydrolyzing enzymes which cleave proteins into peptides and amino acids. A dynamic equilibrium exists between proteolysis and synthesis which is mainly responsible for protein turnover and homeostasis in any tissue [15].

The elevation in the activity levels of glucogenic aminotransferases (AAT & ALAT) in all the tissues during deltamethrin toxic condition suggest their probable mobilization towards gluconeogenesis to meet the energy demands. Elevation in the activity levels of branched chain aminotransferases in all the tissues during deltamethrin toxic state suggests augmented recycling of carbon skeleton of these free amino acids forming respective ketoacids [16]. The elevated branched chain aminotransferases may also be responsible for the generation of series of products essential for fatty acid bio-synthesis and TCA cycle operation [17].

The catabolism of BCAAs such as leucine, isoleucine and valine is initiated by respective branched chain amino transferases (BCAT) resulting in glutamate and corresponding branched chain-keto acids [18]. It was reported that the complete oxidation of BCAAs yields more ATP per mole than the oxidation of glucose, acetoacetate or hydroxyl butyrate [19]. The elevation in BCAT activities observed in the present study indicates the possible impairment in conservation of essential amino acids and also suggests the augmented recycling of the carbon skeleton of these amino acids through respective keto acids [20,21]. It is also possible that the elevated BCATs results in the production of a series of products like acetoacetic acid, acetyl Co-A, succinyl Co-A and propynyl Co-A from BCAAs and contribute these metabolites to either TCA cycle and / or to fatty acid biosynthesis[22]. The values of Total proteins (TP), Soluble proteins (SOLP), Structural proteins (STRP) Values are expressed in mg/g wet wt of the tissue; Free amino acids (FA) Values are expressed as mg of tyrosine equivalents/gm. wet. wt. of tissue; AAT, ALAT Values are expressed as µ moles of pyruvate formed /mg protein / hr; LAT, ILAT & VAT were represented as µ moles of keto acid formed /mg protein / hr).

Table 1. Alterations in the proteins and aminotransferases in Liver of *Ciprinus carpio* during deltamethrin toxic condition

Liver	Control	5ppm	10ppm
TP	115.660	97.668*	91.874
	±1.038	±2.250	±1.775
		(-15.55)	(-5.93)
SOLP	73.987	56.174*	51.935*
	±1.502	±2.911	±1.175
		(-24.07)	(-29.8)
STRP	77.951	67.806*	47.462*
	±1.421	±1.297	±1.042
		(-13.01)	(-39.11)
FA	74.696	91.326*	112.349*
	1.123	1.496	1.378
		(22.26)	(50.4)
AAT	1.729	1.767	2.259
	±0.065	±0.108	±0.037
		(2.18)	(30.65)
ALAT	1.299	1.489*	1.939*
	±0.054	±0.229	±0.050

		(14.62)	(49.24)
LAT	0.904	1.261*	1.320*
	±0.046	±0.021	±0.107
		(39.54)	(46.07)
ILAT	0.828	0.880	1.057*
	±0.034	±0.037	±0.093
		(6.36)	(27.64)
VAT	0.915	1.263*	1.405*
	±0.051	±0.034	±0.246
		(38.09)	(53.63)

All the values are mean, ±SE of six individual observations. Values in '( )'parentheses are % change over Control control.
*Values are significant at P < 0.05 in Scheffe test.

Table 2. Alterations in the proteins and aminotransferses in Heart of *Ciprinus carpio* during deltamethrin toxic condition

Heart	Control	5ppm	10ppm
TP	111.099	99.222*	79.205*
	±21.250	±11.705	±1.775
		(-10.69)	(-20.17)
SOLP	71.160	54.901*	49.073*
	±1.610	±1.754	±3.683
		(-22.84)	(-31.03)
STRP	75.140	59.883*	42.982*
	±1.384	±0.389	±0.792
		(-20.3)	(-42.79)
FA	70.108	83.495*	107.519*
	±1.825	±1.543	±0.961
		(19.09)	(53.36)
AAT	1.272	1.287	1.558*
	±0.010	±0.151	±0.177
		(1.19)	(22.47)
ALAT	1.004	1.242*	1.480*
	±0.114	±0.078	±0.200
		(23.67)	(47.36)
LAT	0.738	0.811*	0.956*
	±0.014	±0.068	±0.028
		(10.01)	(29.62)
ILAT	0.738	0.856*	1.131*
	±0.061	±0.013	±0.143
		(15.96)	(53.27)
VAT	0.732	0.941*	1.142*
	±0.014	±0.020	±0.014
		(28.48)	(55.94)

All the values are mean, ±SE of six individual observations. Values in '( )'parentheses are % change over Control.
*Values are significant at P < 0.05 in Scheffe test.

Table 3. Alterations in the proteins and aminotransferses in Kidney of *Ciprinus carpio* during deltamethrin toxic condition

Kidney	Control	5ppm	10ppm
TP	107.675	87.428*	84.850
	±2.252	±4.899	±1.775
		(-18.80)	(-2.94)

SOLP	60.001	52.118*	46.705*
	±1.478	±1.296	±1.383
		(-13.13)	(-22.15)
STRP	70.157	53.213*	35.094*
	±0.529	±0.248	±0.165
		(-24.15)	(-49.97)
FA	65.036	69.542	84.446*
	±1.638	±1.620	±1.549
		(6.92)	(29.84)
AAT	0.937	0.954	1.188*
	±0.034	±0.032	±0.076
		(1.79)	(26.83)
ALAT	0.674	0.728	1.028*
	±0.072	±0.056	±0.150
		(7.98)	(52.49)
LAT	0.635	0.729*	0.989*
	±0.015	±0.013	±0.363
		(14.79)	(55.71)
ILAT	0.696	0.773*	1.020*
	±0.033	±0.097	±0.192
		(11.01)	(46.48)
VAT	0.470	0.646*	0.756*
	±0.040	±0.026	±0.021
		(37.41)	(60.88)

All the values are mean, ±SE of six individual observations. Values in '( )'parentheses are % change over Control.
*Values are significant at $P < 0.05$ in Scheffe test.

Table 4. Alterations in the proteins and aminotransferases in Brain of *Ciprinus carpio* during deltamethrin toxic condition

Brain	Control	5ppm	10ppm
TP	81.328	76.947	67.84*
	±1.972	±2.048	±1.775
		(-5.38)	(-11.83)
SOLP	48.647	40.764*	35.351*
	±1.478	±1.296	±1.383
		(-16.2)	(-27.33)
STRP	65.078	51.920*	34.639*
	±0.792	±0.774	±1.026
		(-20.21)	(-46.77)
FA	41.345	58.120*	73.828*
	±2.848	±1.187	±2.114
		(40.57)	(78.56)
AAT	0.718	0.820*	0.979*
	±0.040	±0.110	±0.023
		(14.18)	(36.33)
ALAT	0.601	0.699*	0.819*
	±0.069	±0.071	±0.080
		(16.33)	(36.27)
LAT	0.384	0.485*	0.569*
	±0.060	±0.099	±0.137
		(26.25)	(48.09)
ILAT	0.521	0.686*	0.777*

	±0.117	±0.079	±0.128
		(31.7)	(49.2)
VAT	0.454	0.625*	0.680*
	±0.036	±0.053	±0.102
		(37.69)	(49.92)

All the values are mean, ±SE of six individual observations. Values in '( )'parentheses are % change over Control.
*Values are significant at $P < 0.05$ in Scheffe test.

Fig 1. Overview of amino acid metabolism

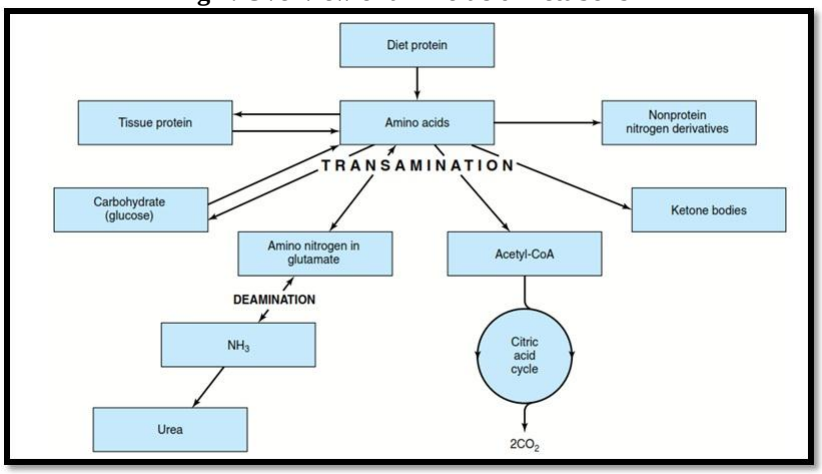
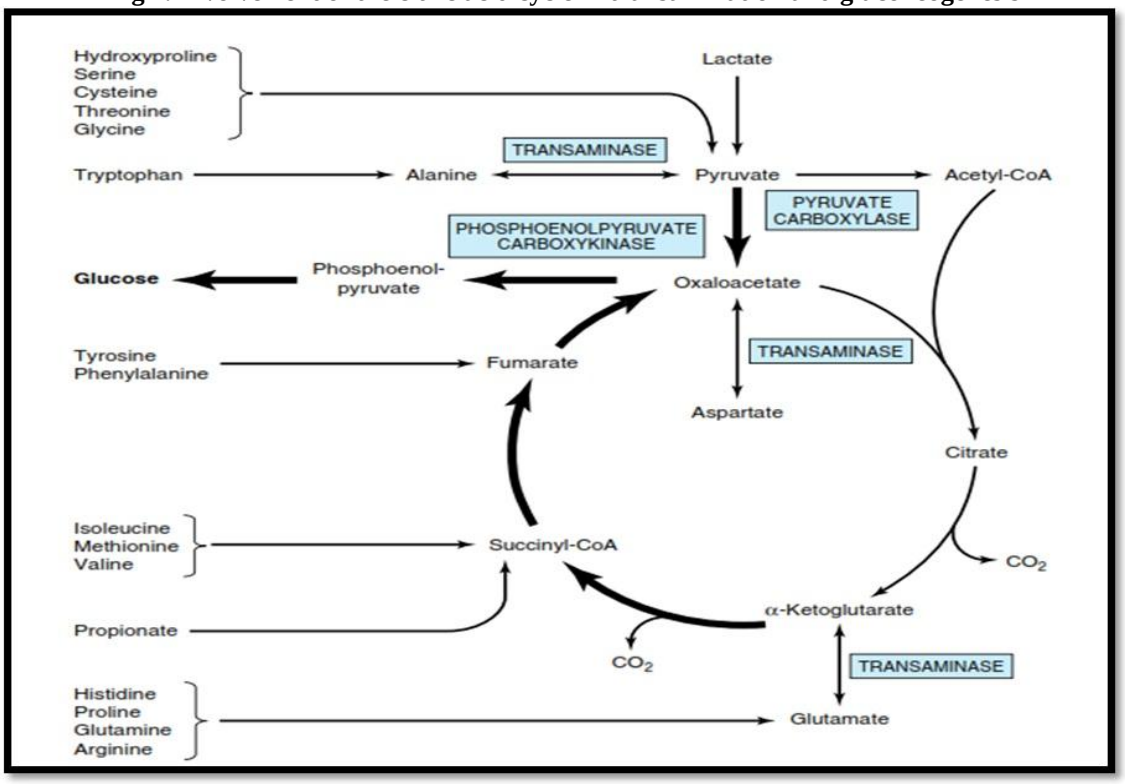


Fig 2. Involvement of the citric acid cycle in transamination and gluconeogenesis



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CONFLICT OF INTEREST

No interest

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