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Analysis of Amino Acids of the Brain in Embryo and Adult of Quail

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Abstract:

Amino acids have the role in the process of proteins synthesis. They are an essential source of nitrogen atoms that have a role in the pathways of synthetic reaction pathways. The carbon skeletons of the amino acids are the source of energy in addition to their role as precursors in the paths of interactions.

The amino acids analysis for the brain of the quail bird in different stage of development (10-16 days of incubation) in addition to the hatching stage (17th day) and the adult.

Materials and Methods:

Amino Acids Analysis

The amino acids were separated from the embryos and adult brains of the quail bird Coturnix coturnix and were diagnosed based on standard amino acids, using high performance liquid chromatographic device (H.P.L.C.).

Results:

The results revealed that there are 16 amino acid: Aspartic acid, Glutamine, Serine, Histidine, Threonine, Glycine, Arginine, Alanine, Tyrosine, Cysteine, Valinine, Methionine, Isoleucine, Phenylalanine , Lysine, and the highest concentration of amino acid at age of [(13,13) days of incubation, adult, (13, 13, 16) days of incubation, adult, (13) days of incubation, adult, (13, 13, 11, 13, 13) days of incubation, adult, (13) days of incubation] respectively and the low concentration at age of [(14, 12, 10, 10,12,10,10,10,10, 14, 12, 12, 15, 10, 14, 10) days of incubation respectively].

Conclusions:

It concluded from this that there was found 16 amino acid that were analyzed in the brains of the quail embryos at the stage (10-16) days of incubation and hatching stage (17) days of incubation and adult (Asp, Glu, Ser, His, Thr, Gly, Arg, Aln, Tyr, Cys, Val, Met, Ili, Leu, Phe, Lys, and Lys) the highest concentration of aging (13, 13, adult, 13, 13, 16, adult, 13, 13, 11, 13, 13, adult and 13) respectively, and less concentrated in ages (14, 12, 10, 10, 10, 10, 14, 12, 12, 15 10, 14, 10) days of incubation, respectively.

Keywords: Amino acids analysis,brain,quail,embryo,adult.

Introduction:

The amino acids are the basic installation unit of the proteins, which exist in nature over 20 amino acids, and all containing the carboxyl group and amino group are connected to the carbon atom. All amino acids contain a symmetrical carbon atom (except for glycine) in the form of D or L. In general all the amino acids that are naturally found in the tissues are formed as L. The acids are produce from hydro-protein and a part of which is freely found in the tissues of the body, and plays a role in the intermedia processes. Amino acids have the role in the process of proteins synthesis. They are an essential source of nitrogen atoms that have a role in the pathways of synthetic reaction pathways. The carbon skeletons of the amino acids are the source of energy in addition to their role as precursors in the paths of interactions (1). The amino acids are divided into two basic types, essential amino acids (AA), including Isoleucine (Ile), Leucine (Leu), Lysine (Lys), Methionine (Met), Phenylalanine (Phe), Threonine (Thr), Tryptophan (Try) and Valine (Val), which are obtained by the living organism through food. Mammals lack the complex and long pathway of interaction necessary for their synthesis. While the second type of amino acids are non-essential amino acids (NAA) includes Alanine (Ala), Arginine (Arg), Aspartate (Asp), Cysteine (Cys), Glutamine (Glu) and Glycine (Gly), Histidine (HIS), Proline (Pro), Serine (Ser) and the Tyrosine (Tyr), which are synthesized by living organisms mediated simple reaction pathways, either from the pyruvate, oxaloacetate, α-ketoglutarate or glycerate-3-phosphate except the Tyrosine acid (Tyr) which is derived from phenylalanine amino acid (Phe) (2, 3). The amino acids are divided into six families, with each family of the same precursor molecule, the glutamate family, which includes the amino acids (Glu), Proline (Pro) and Arginine (Arg). The family of the Serine including Serine (Ser), Glycine (Gly), Cysteine (Cys) and the Aspartate include the Aspartate amino acid (Asp) only. The Pyruvate family includes the Alanine (Ala), Valine (Val), Leucine (Leu) and Isoleucine (Ile), including the family of histidine amino acid (His), which is the main amino acid that enters the synthesis of enzyme-catalyzed for interactions in the cells organism. The aromatic family includes Phenylalanine (Phe), Tyrosine (Tyr) and Tryptophan (Try) (1, 4, 5). The eukaryotic cells have a large number of enzymes that are carriers of the aminotransferase as they are located inside the cytoplasm and mitochondria, and that these enzymes possess two properties; the first depends on the amino acid type α-amino acid which give α-amino group and the second α-keto acid that receives α-amino group (6).

It has been found that about 50% of the fertilized eggs of the human being are advanced to the stage of the blastocysts stage in the media containing the amino acids and the eggs die when they are in a free-of-the-amino-acids media (7). There is a discrepancy in the amino acids in the different species, as the Glycine amino acid (Gly) has high concentrations in most species except for humans (3, 8). The Tyrosine amino acid (Tyr) is an aromatic amino acids that affect the synthesis of dopamine, which contributes to the building of the neurotransmitter norepinephrine, thus increasing the level of Tyrosine (Tyr) means increasing the rates of dopamine and norepinephrine (single amines), which affect the condition of hyperactivity in the organism (9). Nellin and Cook (10) pointed out that the head of the final amino acids series n-terminal amino acids alpha and beta lipovitellinin (α-and β-lipovitellinin) are the two amino acids (Arg) and (Lys). The protein (α-Laminin) is a glycoprotein non-glycogenic protein naturally formed in the base plate and has a role in the closing of the neural tube, and when its absent in the mutant mice leads to the failure of closing the anterior neuropore (exencephaly) (11).

The tyrosine kinases and their receptors tyrosine kinases play an important role during the migration and differentiation of the nerve cells, and the direct direction of their axons inside the nervous system (12,13). Watkins (8) confirmed that the amino acid (Gly) works to minimize the damage of inorganic electrolytes during the embryogenesis stages in the rat. The chain of amino acids in neurohormones in the brain differs from the sequence of amino acids in the hormones of the pituitary gland in the rat (14) Whereas in the chick, the amino acids sequence in neurohormone are identical to the hormones of the pituitary gland. (15, 16). During the embryogenesis stages, the growth hormones were found in the brain of the chicken and rats embryo (17). The amino acid (Arg) decreases in the telencephalon and diencephalon in the chicken at the stress and fasting as well as the effect of the high degree of environmental temperature on the amino acids concentrations in the brains of young chickens. The temperature rises effect on the free amino acids concentrations in the diencephalon, due to the fact that the raised of environmental temperature leads to increased damage in the diencephalon which causes a change in the concentration of some of the free amino acids in the diencephalon and plasma and the change in the metabolism of the amino acids contributes to the damage in the chicken brains. The heat receptors are located in the hypothalamus in the brain, so this part of the brain can be more affected during heat exposure which affects the behavior (6, 18).

The neutral-cellular adhesion molecules (N-cam) are mediated by congenital birth events as they have an essential role in the formative organization and that the morphological differences of the nervous system in the animals of the same species as well as in the different species and belong to differences in the expression gene for molecules (N-cam). These molecules have been found in human embryos, mice, rats, chickens and frog were marked by the mediation of the single monoclonal antibodies and it contains the sialic acid rich in carbohydrates and that the series of amino acids have similar types and are found during the period of histogensis (19).

The current research objective is to identify the concentration of amino acids in the brains of the quail embryos (10, 11, 12, 13, 14, 15, 16, 17) days of incubation as well as adult.

Materials and Methods:

Amino Acids Analysis

The amino acids were separated from the embryos and adult brains of the quail bird Coturnix coturnix and were diagnosed based on standard amino acids, using high performance liquid chromatographic device (H.P.L.C.), the device was injected with a 1mM/L for each standard amino acid as the conditions of separation described in the list below:

Column long	C18 (150×4.6) (5.0 µm)
Flow	2.0 Ml/min
Detector type	Fluorescence
Wave length	Shimadzu
Temperature	25 °C
Carrier phase	A: Buffer Na_2HPO_4 B: Mix = $H_2O.CH_3CN.MeO_4$

Methods:

Method of work based on the method of Schuster (18).

A homogenous (10-20) mg of brain was used by hand homogeniser and then added to the homogeny 0.5 cm3 of perchloric acid.Samples were centrifuged at a speed of 4000 rpm for 10 minutes.250 µl of supernant was added to potassium hydrogen carbonate.The mixture solution was blended and placed in a snowy bath for 15 minutes, the mixture solution was blended again then centrifuged for 10 minutes and quickly 6000 rpm.250 µl of the clear was added to 375 µl of the diluted (Li Citrate) solution with a concentration of (0.2 M) (pH 1.30).The amino acids were measured by using a high performing liquid chromatography device (HPLC).The calculated concentration of the amino acids using the following equation:

Concentration of amino acid (mM/L) =(sample in acid space pack)/(standard acid space pack)×standard concentration

Results:

The results of the analysis of amino acids in the brains of the quail embryos for stages (10, 11, 12, 13, 14, 15, 16) days of incubation and hatching stage (17) days, the adult (Figure 1) that there are (16) amino acid (table 1). The highest concentration was the following amino acids (Asp), (Glu), (His), (Thr), (Ala), (Cys), (Val), (Ile), (Leu) and (Lys) were in embryo (13) days of incubation, reaching concentrations as follows (0.128, 0.093, 0.098, 0.076, 0.056, 0.078, 0.078, 0.083, 0.084, 0.094) mM/L respectively (Figure 2). The result also indicated that amino acid (Ser), (Arg), (Tyr) and (Phe) were increasingly concentrated in the adult brain of the bird, as follow (0.064, 0.103, 0.123, 0.098) mM/L respectively.Methionine (Met) had the highest concentration in the brain of the embryo age (11) days of incubation which reached (0.078) mM/L while (Gly) had highest concentration in embryo at (16) days of incubation age (0.028) mM/L (table 1) (Figure 2).

It was also noted that there was a decrease in the concentration of a number of amino acids in the brain of embryo at (10) days of incubation, which are (Ser), (His), (Arg), (Ala), (Tyr), (Leu) and (Lys) (0.025, 0.036, 0.018, 0.042, 0.021, 0.043, 0.031, 0.035) NM/L respectively. The result also found in embryo at (12) days of incubation of the lower concentration of the following (Glu), (Thr), (Val) and (Met) have dropped their concentrations (0.036, 0.041, 0.040, 0.052) mM/L respectively.

In embryo at 14 days of incubation, the amino acids such as (Asp), (Cys) and (Phe) were of low concentration and reached (0.032, 0.038, 0.051) mM/L respectively, while the amino acid (Ile) decreased its concentration and reached (0.046) mM/L in embryo at 15 days (Table 1).

Table (1): The concentrations of amino acids in the brains of the quail embryos at ages (10, 11, 12, 13, 14, 15, 16, 17) Days of aging.

Ages / Amino acid mM/L	(10) days	(11) days	(12) days	(13) days	(14) days	(15) days	(16) days	(17) days	Adult
(Asp) Aspartic acid	0.04516	0.06518	0.06311	0.12833	0.03278	0.03327	0.05081	0.06902	0.014228
(Glu) Glutamine	0.03753	0.07807	0.03685	0.09384	0.04018	0.0536	0.05613	0.07069	0.08487
(Ser) Serine	0.02562	0.05941	0.03435	-	0.03222	0.0283	0.04363	0.05425	0.06456
(His) Histidine	0.036392	0.08436	0.05744	0.09880	0.04756	0.04656	0.06108	0.07467	0.09243
(Thr) Threonine	-	0.06356	0.04109	0.076251	0.03683	0.0444	0.04416	0.05805	0.07068
(Gly) Glycine	0.01819	-	-	-	-	0.0269	0.02869	-	-
(Arg) Arginine	0.04227	-	-	-	-	0.0621	-	-	0.10349
(Ala) Alanine	0.02149	0.04799	0.03131	0.05600	0.02740	0.0327	0.03505	0.04374	0.05295
(Tyr) Tyrosine	0.04364	0.09574	0.06111	0.11907	0.05598	-	-	0.09154	0.12370
(Cys) Cysteine	-	0.06590	0.04217	0.07867	0.03801	-	0.04730	-	0.07272
(Val) Valine	-	-	0.04077	0.07802	-	0.0409	0.04583	-	-
(Met) Methionine	-	0.07861	0.05229	-	-	-	0.05680	-	-
(Ile) Isoleucine	-	0.06990	-	0.08380	-	0.0468	0.05061	-	-
(Leu) Leucine	0.03195	-	0.04529	0.08405	-	-	-	-	-
(Phe) Phenylalanine	-	0.08743	-	-	0.05178	-	-	0.08053	0.09848
(Lys) Lysine	0.03561	0.06898	0.05048	0.09433	0.04582	0.0524	0.05593	0.071143	0.08752

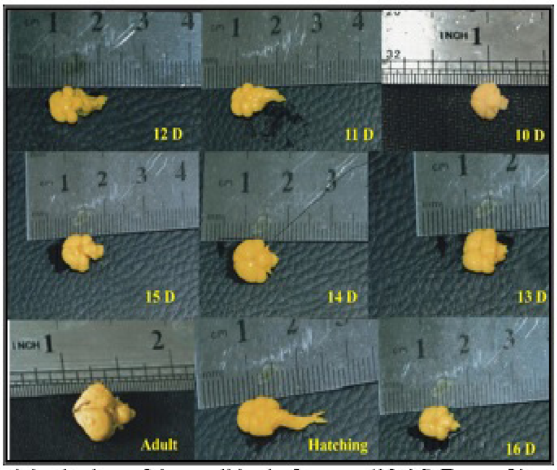

Figure (1): A back view of the quail brain for ages (10-16) Days of incubation and hatching stage and adult

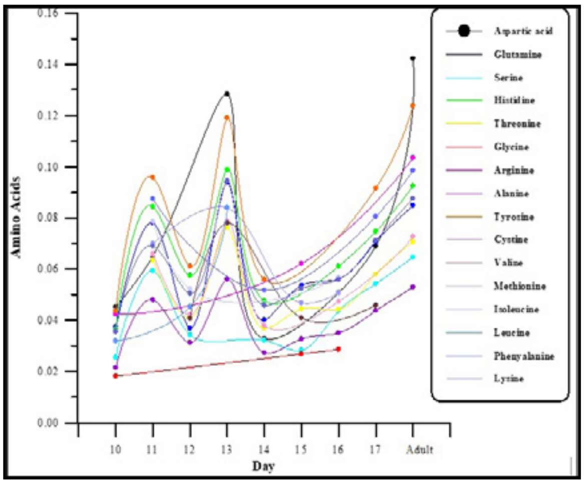

Figure (2): Illustrates the relationship between amino acids in the brains of the quail embryos for ages (10-16) days of incubation and hatching (17) days of incubation and adult

Discussion

The amino acids play an essential role in the vital activities of the body and also participate in the neuro transmitter systems (20, 21, 22). The formation of neurosynapses in large numbers in the central nervous system needs to amino acids, acting as neurotransmitters in the brain and the spinal cord, the Purkinje cells in the cerebellum, build amounts of amino acids during the different stages of embryogenesis (23, 24).

Fuchs *et al*. (25) indicated that the amino acid (Asp) has a role in embryogenesis processes and in endocrine events as well as Sato *et al.* (26) were indicated that during the incubation period of the chicken the concentration of amino acids (Asp) and (Glu) are high indicating that the composition the central nervous system in embryo chickens requires large amounts of this amino acids and this corresponds to the outcome of the current study as there were increase in their concentrations, especially at age 13 days of incubation.

The two amino acids (Asp) and (Glu) also represent excitatory neurotransmitters in the brain and spinal cord in the vertebrates, while the amino acid (Gly) is a inhibitory neurotransmitter, as it acts to open portal channels associated with the chlorine ion, and have a role in making a concentration difference so it helps spread it into the nerve cell and as ion chlorine is a negative charge, so crossing it makes the inner-side charge of the cell membrane more negative in case of rest, and it's called a more polarized effort after inhibited synapse this is important in controlling the movements of the body and on the functions of the brain (27, 28).

Fisher (7) indicated that the amino acids containing two groups of carboxyl, such as (Glu) and (Asp) have an important role in metabolic processes that occur in the brain, which stimulating the (Glu) to increase the rate of nerve cells composition in the cerebral cortex and also conceder as dominated neurotransmitter in the brain, as it is associated with the receptors that are located on the surface of the nerve cells in neuropostsynaptic caused a change in the function of the canals associated with the plasma membrane as it increases its permeability of sodium and calcium ions causes the plasma membrane depolarization so they found their concentration in this result during the embryogenesis were the highest for glutamine acid 0.093 mM/L and acetic acid in embryo (15) day of incubation while the adult the highest concentration ratio for (Glu) acid and (Asp) and this may support their important role in metabolic processes.

The first beginning of the efficacy of embryonic organs in the chicken embryo occurs at different stages of embryonic maturity. The beginning of the heart effectiveness occurs at the day (2) from the incubation. The brain and the eye occur at the day (10 and 11) and there is a relationship between the effectiveness of the organs and the concentration of the amino acids like (Glu) (29), which does not correspond to the results of the current study where the concentration of amino acid (Glu) was high in embryo(13) days of incubation.

Studies have indicated that amino acid (Glu) plays an organized role in the propagation of the precursor of neurons in the fore brain (6, 30, 31), as there is (Glu) in the brain-generated areas of the telencephalon, which is an important role in the morphogenesis of neurons and the regulation migration and differentiation (4, 8, 32). The primary neurotransmitter (Glu) is highly effective when it is highly concentrated and represents an important role in the pathogenicity of the central nervous system and this applies with the current result (8).

The brain is a significant source of glutamine synthase enzyme, and is sensitive to the toxicological effects of ammonia, as brain cells work to convert ammonium to (Glu), which is a non-toxic molecule and then (Glu) transfers to the liver that represents the disposal place from nitrogen waste. (Ser) is important in the composition of the nervous system so the synthesised efficacy is a high during embryogenesis and the inhibition of (Ser) synthesis that produces mental disabilities (26, 33) and in this result was found it has a high concentration in the brains of quail embryos at ages (11, 12, 14, 15, 16 17) days of incubation compared to the age of 10 days of incubation

as well as Patzold *et al.* (34) showed that the presence of (Ser) in the brains of the chicken Gallus domesticus and Anser anser adult, this corresponds to the results of the present study as it was the highest concentration in the adult brain of the bird. Hamase *et al.* (35) indicated that (Ser) exists in many areas of the brain except the cerebellum and the medulla oblongata and has an important and essential role in the differentiation of nerve cells, and in the metabolic processes of the amino acids, as it is relevant in stimulating the efficacy of the future of n-methyl-d-aspartate receptor (NMDAR), as the change of function of that future leads to schizophrenia and epilepsy (25, 34).

The current result also found that (His) resides in the embryos at age (10, 11, 12, 13, 14, 15, 16) day of incubation and adult. (His) is the precursor of histamine and neurotransmitters play an important role in generating the microchemical environment of the nerve cells especially in the early stages of embryogenesis, which have specific receptors for neurotransmitters on the surfaces of the cells which are represented neural stem cells present in the brain areas. The neurotransmitter have a role in the regulation of nerve cell multiplication, immigration, differentiation and preservation of the precursors of nerve cells (36, 37).

It has been found that an organic acid taurine which is a component of the plasma membrane of cells, organizes the transport of food through the cell membrane and also works to protect cells from toxins in addition to it acts as a neurotransmitter, boosts muscle contraction and activates the heart muscle as well as reduces blood pressure. The taurine synthesis process is done of (Cys) by cysteine-sulfinate decarboxylase (CSD), which was found in chicken embryos that the enzyme begins to appear after (12) hours of incubation (38, 39), in the present finding, the increased concentration of (Cys) was in embryo (13) hours of incubation.

Vangelder and Belanger (2) through their study of the amino acids in chicken embryos indicated that the embryonic period (E2-E4), from the age of (6.7 to 9-18) hours of incubation the concentration of the (Val) and (Glu) are high in the embryo, while in the period (E4-E7), the age of (9-18) hours to (23-26) hours of incubation. The concentration of the (Val) is reduced in the brain, then its concentration gradually begins to decrease as well as in the period (E9-E15) (i.e., 29-33 to 50-55) hours of incubation. The concentration of (Glu) at all periods was three times more than the concentration of the (Val), and there was an increase in the concentration of (Val) in embryo (13) days of incubation and less concentration at age (12) days of incubation. When compared with (Glu) was found to be the most concentrated of it at the age of (13) days of incubation and its lowest concentration was at the age of (12) days of incubation.It concluded from this that there were found 16 amino acid analyzed in the brains of the quail embryos at the stage (10-16) days of incubation,hatching stage (17) days of incubation and adult (Asp, Glu, Ser, His, Thr, Gly, Arg, Aln, Tyr, Cys, Val, Met, Ili, Leu, Phe, Lys, and Lys) the highest concentration at aging (13, 13, adult, 13, 13, 16, adult, 13, 13, 11, 13, 13, adult and 13) respectively, and least concentration at ages (14, 12, 10, 10, 10, 10, 14, 12, 12, 15 10, 14, 10) days of incubation, respectively.

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