Bionatura
Latin American Journal of Biotechnology and Life Sciences

Bionatura Issue 3 Vol 8 No 1 2023

Article

Estimation of amino acid concentrations in the kidney of albino mice embryo

Amar Hussein Abd Ali, Nahla A. Al-Bakri*
Department of Biology / College of Education for Pure Science (Ibn Al-Haitham) / University of Baghdad, Iraq.
*Correspondence:nahlaalbakri@yahoo.com
Available from: http://dx.doi.org/10.21931/RB/CSS/2023.08.03.54

Abstract
Amino acids are the basic building block for peptides and proteins. They are raw materials for generating hormones, purines, pyrimidines and vitamins. Amino acids also provide the body with energy through their carbon structures. The study analyzed the amino acid in the kidneys of the albino mice embryo at 17 and 19 gestation days, using a high-performance liquid chromatography device (HPLC). Samples were obtained after removing them from the embryo and placing them in an ice bath to prevent cell lysis and acid loss. The study found 18 amino acids in the kidneys of the albino mice embryo. They are Asparagine (Asn), Glutamine (Glu), Serine (Ser), Glycine (Gly), Threonine (Thr), Histidine (His), Cysteine (Cys), Alanine (Ala), Proline (Pro), Tryptophan (Tryp), Arginine (Arg), Tyrosine (Tyr), Valine (Val), Methionine (Met), Isoleucine (Ile), Leucine (Leu), Phenylalanine (Phe), and Lysine (Iys).

Keywords: Amino acid, *Mus musculus*, Embryo, Kidney.

Introduction

Amino acids are organic molecules representing proteins and peptides' structural and functional units. Likely, Amino acids play a primary role in energy molecules, antioxidants, and electron carriers. Amino acids exist in humans and animals. They are chemically composed of an amino group NH2 and a carboxyl group COOH, linked to a central carbon atom and an R-side chain. Theoretically, there are a large number of amino acids that can be synthesized, but biologically, there are only 20 amino acids. Most acids are found in the body in the form of the letter "D" [1, 2]. The organism's body synthesizes half of the amino acids. The essential acids are the amino acids the organism obtains through food; they include (His, Ile, Lys, Leu, Met, Phe, Trp, Thr, and Val). The non-essential amino acids include (Ala, Arg, Asp, Cys, Glu, Gly, Ser, and Tyr). The organism body gets them from other sources [3, 4].

Essential amino acids (EAAs) and non-essential amino acids (NAAs) play a fundamental role during the development mouse at different times and by different mechanisms. Non-essential amino acids, for example, increase the completion of cell division during mitosis, whereas the essential amino acids support developmental embryo [1]. Arginine and leucine are the two most important amino acids that are key in regulating development and differentiation, especially in the blastocyst stages [5, 6]. Arginine regulates the formation and movement of the layer Trophoectoderm that interacts with the uterus during implantation [7]; Arginine also helps regulate and function mitochondria to become more stable [1]. The kidney has an essential role in the metabolism of amino acids by regulating the concentration

of many amino acids in the plasma. The kidney also maintains the state of homeostasis through the processes of synthesis, filtration, reabsorption, and excretion of urea [8]. The kidney is the main organ for getting rid of Proline and Glutamine from venous blood. It also produces amino acids such as Arginine, Tyrosine and Serine; it then exports them to other tissues [9]. Previous studies on mice indicated that the kidney tubules, especially the proximal convoluted tubules, reabsorb amino acids from the urinary filtrate and prevent their loss with urine [10, 11, 4, 12].
This study aims to estimate the concentration of amino acids in the kidney of the *Mus musculus* embryo at the age of 17 and 19 days of gestation.

Materials and Methods

Animal breeding

This study was conducted on *Mus Musculus* embryos of the type Albino mice. Adult female and male mice were obtained from the Al-Razi Center / Industrial Research and Development Authority and the National Center for Drug Control and Research. The age of mice was approximately (8-10) weeks [13].
Vaginal smears were made for female mice to identify the type of estrous cycle to prepare them for mating with males [34]. One adult male and two to three sexually mature females were placed in the cage at night and left until the early morning of the next day to ensure that mating occurred by noticing the vaginal plug at the vaginal opening or watching the sperm in the vaginal smear [14, 15].

Obtaining embryos

The pregnant mother is sacrificed after being anesthetized, and at the midline of the body, a longitudinal incision was made from the outlet area to the sternum by dissection scissors, and the skin and peritoneum were removed. Then, the embryos were extracted from the surrounding covers by the cervical dislocation of Uterus [16].

Extracting amino acids

The obtained kidney samples from the embryo are placed in an ice bath to prevent cell lysis and loss of amino acids [17].

Amino acid analysis

Amino acid analysis was performed on albino mouse embryos based on amino acid standard models. For this purpose, a high-performance liquid chromatography (HPLC) device was used. The device was injected with a known concentration (20µL) of each standard amino acid under the following separation conditions:

Wavelength	Shimadzu
Carrier phase	A: Buffer
	B: Methanol
Detector-type	UV set at 254 nm
Column long	C18
Temperature	25°c

The method of amino acid analysis was according to [36] as follows:

- Crushing (20-50) mg of embryo kidney tissue at 17 and 19 days of gestation and humanizing it with (20) ml of deionized water. Then, 1.5 g of sulfosalicylic acid was added. All compounds are mixed using Vortex and left for an hour.
- Samples were centrifuged for 1 hour in a Centrifuge device and filtered through a 2.5 µm filter.

- Hydrochloric acid (HCL) was added at a concentration of (0.1N) to make the supernatant acidic (pH 2.0), then centrifuged at a speed of (3000) r/min.
- Mixing (10) µL of the sample extract with (10) µL of Phenyl isothiocyanate (PITC) after one minute, then adding 50 µL of sodium acetate (0.1) (pH 7.0).
- Shaking the sample using ultrasound for 10 minutes, then filtering the sample using 0.2 µm filters.
- 20 µl are injected into the column of an HPLC liquid chromatograph, and the concentration of each acid is quantitatively determined by comparing the peak area of the standard form with that of the samples.
- The concentration of amino acids is calculated using the following equation:

Amino acids concentration = $\frac{\text{sample in packet area}}{\text{standard packet area}}$ x standard concentration x dilution times number.

Results

The analysis of the amino acids in the kidney of the albino mice embryo, aged (17 and 19) on the day of gestation showed:

There were no significant differences between all the amino acids, individually or totally. The average concentrations in the fetal kidney aged between (17 and 19) gestational days were (1.015 ±0.09and 0.942±0.08), respectively (Table 3-1, Figure 3-1).

The analysis also found that 18 amino acids with concentration in the kidney of the embryo at the age of 17 days of gestation were as follows:

Asn (1.006±0.052), Glu (1.751±0.084), Ser(0.580±0.043), Gly(0.669±0.019), Thr(0.540±0.088), His(1.142±0.438), Cys(0.967±0.355), Ala(1.670±0.319) Pro (1.518±0.218), Tryp(1.008±0.286), Arg(0.805±0.207), Tyr (0.830±0.192), Val(0.803±0.217), Met(0.689±0.220), Ile(0.670±0.164), Leu(1.523±0.126), Phe(0.884±0.129), Iys(1.214±0.095), Table (2-3) Figure (3-3).

In the kidney of the embryo at the age of 19 days of gestation, the concentration of amino acids was as follows:

Asn (1.142±0.194), glu (1.857±0.136), ser(0.628±0.114), Gly(0.674±0.060), Thr(0.508±0.041), His(0.830±0.055), Cys(0.701±0.140), Ala (1.267±0.025), Pro(1.262±0.070), Tryp(0.733±0.097), Arg(1.066±0.129), Tyr (0.653±0.103), Val(0.981±0.153), Met(0.696±0.105), Ile(0.870±0.135), Leu (1.313±0.122), Phe(0.743±0.100), Iys(1.037±0.071), Figure (3-3). Table (2-3)

In this result, it was found that the highest concentration of amino acids in the embryo of 17 gestation days was (Glu) acid with a concentration of (1.751 ± 0.084) and the lowest concentration of (Thr) (0.540 ± 0.088) acid. Similarly, in the embryo of 19 gestation days, (Glu) acid was the most concentrated (1.857 ± 0.136), and (Thr) acid was the least concentrated (0.508 ± 0.041) Fig. (3-4).

Concentration of Amino acid (Mean±S.E)		
Group	**Embryo 17 days of gestation**	**Embryo 19 days of gestation**
Amino acid	1.015±0.09	0.942±0.08

Table (3-1). Concentration of Amino acid in the Kidney of *Mus musculus* embryo.

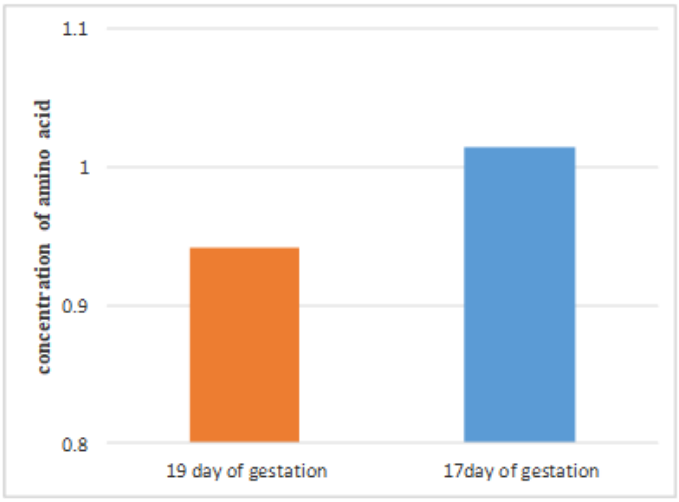
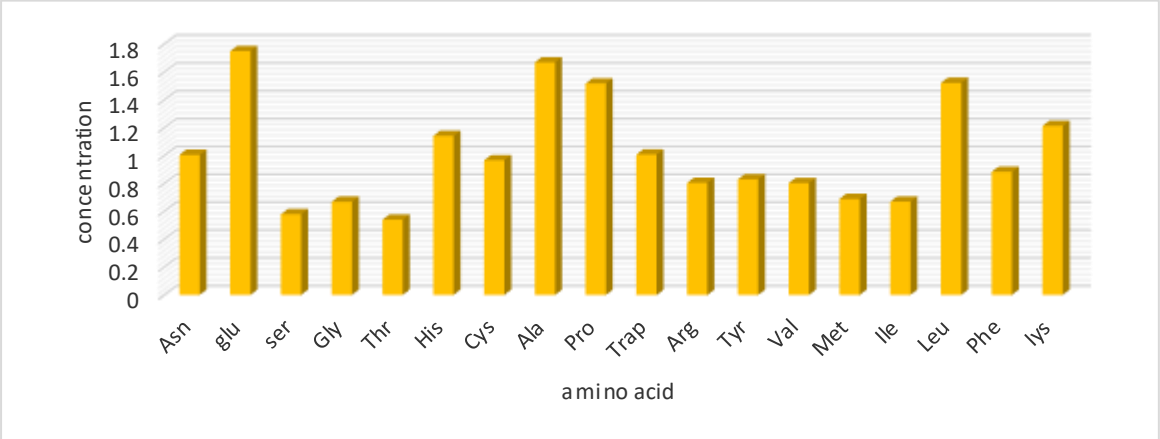


Figure (3-1). Amino acid concentration in the Kidney of Albino Mice embryo is estimated at 17,19 DPC. There are no significant differences in p-value (0.05)

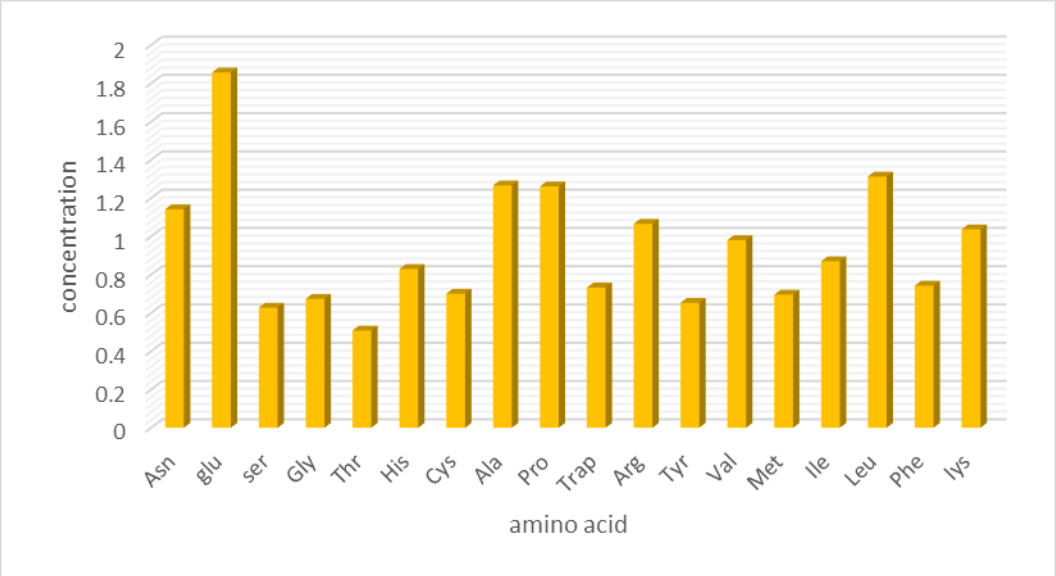
Concentration mg (Mean±S.E)		
Amino acid	**Embryo 17 days of gestation**	**Embryo 19 days of gestation**
Asn	1.006±0.052	1.142±0.194
Glu	1.751±0.084	1.857±0.136
Ser	0.580±0.043	0.628±0.114
Gly	0.669±0.019	0.674±0.060
The	0.540±0.088	0.508±0.041
His	1.142±0.438	0.830±0.055
Cys	0.967±0.355	0.701±0.140
Ala	1.670±0.319	1.267±0.025
Pro	1.518±0.218	1.262±0.070
Tryp	1.008±0.286	0.733±0.097
Arg	0.805±0.207	1.066±0.129
Tyr	0.830±0.192	0.653±0.103
Val	0.803±0.217	0.981±0.153
Met	0.689±0.220	0.696±0.105
Ile	0.670±0.164	0.870±0.135
Leu	1.523±0.126	1.313±0.122
Phe	0.884±0.129	0.743±0.100
Iys	1.214±0.095	1.037±0.071

Table (3-2). The concentration of each Amino acid in the embryo of *Mus musculus* 17 and 19 days of gestation.



F

Figure (3-2). The concentration of each Amino acid in the embryo kidney at 17 days of gestation.



Figure(3-3).The concentration of each Amino acid in the kidney of the embryo at 19 days of gestation

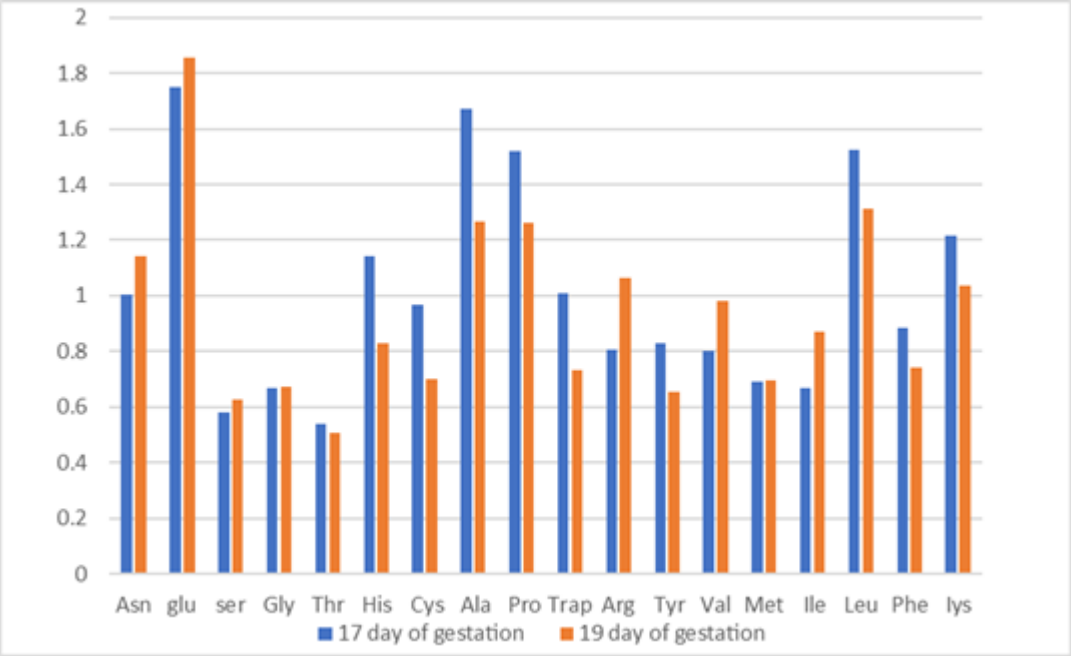


Figure (3-4). The Relationship between Amino acid concentration in the Kidney of *Mus Musculus* Embryo 17 and 19 days of gestation.

Discussion

The results of the current study, which analyzed the amino acids in the embryo of the *Mus musculus* (albino mouse), showed the diagnosis of 18 amino acids. This finding is consistent with [18] in the kidney of the adult mouse 19, found in their study that the number of amino acids in the liver of an adult embryo and adult *Gallus gallus domstica* (domestic chicken) was 18 amino acids. Furthermore, [20] found that the number of amino acids in the brain of an embryo and adult quail (*Coturnix coturnix*) was 16.

This study also found that the highest concentration of acids in the kidney of the albino mouse embryo at the age of 17 and 19 days of pregnancy is the (Glu) acid with a concentration of (1.857 ± 0.136), whereas (Thr) acid was the lowest concentration of (0.508 ± 0.041). On the contrary, [18] indicated that (Tau) acid was the highest concentration acid in the kidney of the adult mouse (508.02 ± 63.40), and the lowest concentration was (Leu)(14.89 ± 1.60). Furthermore, it was found in this study that the concentrations of (Tryp) acids were (1.008 ± 0.286 and 0.733 ± 0.097) respectively and (Cys) acids with a concentration of (0.967 ± 0.355 and 0.701 ± 0.140), respectively in the kidneys of the 17th and 19th days of gestation 21, indicated that (Tryp) and (Cys) was also available in the mouse embryo. The concentration of (Tryp) was (0.05). The concentration of (Cys) was (0.1) [21,] confirming that the (Tryp) and (Cys) acids increase during the development of the embryo, and this is consistent with the study of [32] who also indicated that (Tryp) plays a significant role in building proteins.

[22] explained that the amino acids (Glu Cys, Arg, and Tryp) are functional.

Tryptophan acid is one of the essential amino acids; it plays a significant role in the construction of some vitamins, including vitamin Nicotinamide. In addition to being a neuron transmitter, Tryptophan acid plays a role in inhibiting cellular receptors for inflammation. It can be converted to Serotonin in smooth muscle fibers [23, 24]. [35] indicated that the transfer of all amino acids, including Tryptophan, to the blastocyst stage is necessary for building proteins, protecting the trophoblast layer, and stimulating the synthesis of T-cell proliferation.

Cysteine acid is a non-essential amino acid that the body manufactures. It is vital in

building proteins and the synthesis of collagen. Cysteine is a component of the plasma membrane; it transports nutrients through cells, including sulfur, and is one of the most essential antioxidants and neurotransmitters [25]. Likely, [26] indicated that Cysteine acid contributes to stimulating neural development, including brain formation, in addition to increasing cardiac muscle contraction and activation. Cysteine contributes to the construction of taurine through the enzyme Cysteine-sulphate decarboxylase [27].

Glutamine acid is one of the non-essential amino acids; it is the most prevalent of the rest of the other acids. Glutamine acid enters the building of proteins, sugar acids and nitrogenous bases such as purines and pyrimidines, which leads to the formation of nucleic acids. It likely provides a carbon source through oxidation in some cells [1, 33]. Glutamine synthesizes amino acids like Citrulline, Arginine, Proline, and Asparagines and the energy complex NAD [24].

Glutamine is the largest donor of ammonia NH3 in the kidney. Ammonia is separated from Glutamine by the enzyme phosphate-dependent glutaminase; it then migrates to the lumen of the collecting ducts to combine with the hydrogen ion H+ from carbonic acid HCO3, forming ammonium NH4. It then travels to the renal vein while the rest is excreted with urine [28]. This process is necessary to maintain the pH of the blood plasma [29].

Glutamate acid is derived from Glutamine by the enzyme Renal glutaminase, which is released to the circulatory system. The process of transamination occurs to form alanine and α-ketoglutarate [30, 31]. Accordingly, we find that Glutamine is the most essential acid and the primary reactive substance; this explains its presence in higher concentrations than the rest of the acids in the results of the current study. The finding showed that its concentration in the kidney of the mouse embryo at the age of 17 and 19 days of gestation was (1.751 ± 0.084 and 1.857 ± 0.136) respectively. This finding matches with [19]. They found that Glutamine was the highest concentration in the embryo of 7, 11, 14 and 19 incubation days and adult *Gallus gallus domstica* (domestic chickens). The increased concentration in embryonic ages is due to its importance.

Conclusions

It was concluded that the highest concentration of acids in the kidneys of the Mus musculus embryo, at the age of 17 and 19 days of gestation, is Glutamine acid (Glu), while Threonine acid (Thr) was the lowest concentration in the embryo.

References

1. Leese, H. J, McKeegan, R. PJ and Sturmey, R. J. Amino Acids and the Early Mammalian Embryo: Origin, Fate, Function and Life-Long Legacy. *Int. J. Environ. Res.*, **2021**; *18(18),* 1-19.
2. Wu, G. Amino Acids Biochemistry and Nutrition. 2nd ed. CRC Press. **2022**.
3. Chianese, A., Franci, G., Galdiero, M…. Stelitano, D. Amino Acids, Peptides and Proteins. 43th ed. The Royal Society of Chemistry. London. **2019**.
4. Makrides, V., Camargo, S. M. and Verrey, F. Transport of amino acids in the kidney. *Com. Physiol.*, **2014**; *4(1):* 367–403.
5. Bröer, S. and Bröer, A. Amino acid homeostasis and signalling in mammalian cells and organisms. *Biochem. J.*, **2017**; *474:* 1935–1963.
6. Milanesi, R.; Coccetti, P. and Tripodi, F. The regulatory role of key metabolites in the control of cell signaling. Biomolecules, 10, 862. **2020**.
7. Bazer, F. W, Johnson, G.A. and (Wu, G.). Amino acids and conceptus development during the peri-implantation period of pregnancy. Cell Signal. Dur. Mamm. *Early Embryo Dev.*, **2015**; *843,* 23–52.
8. Young, G. A. Amino acids and the kidney. *Amino Acids,* **1991**; *1(2),* 183–192.

9. Kopple, J.D. Phenylalanine and tyrosine metabolism in chronic kidney failure. *J. Nutr.*, **2007**; *137*:1586-1590.
10. Chan, Y. L., and Huang, K. C. Microperfusion studies on renal tubular transport of tryptophan derivatives in rats. *The Am. J. Physiol.*, **1971**; *221(2)*,575–579.
11. Günther, R., and Silbernagl, S. Renal handling of L-histidine studied by continuous microperfusion and free flow micropuncture in the rat. Pflugers Archiv: Europ. *J. physiol*, **1981**; *389(2)*, 137–142.
12. Oken, D. E., and Weise, M. Micropuncture studies of the transport of individual amino acids by the Necturus proximal tubule. *Kidney int.*, **1978**; *13(6)*, 445–451.
13. McCracken, J.M., Jiang, L. and Pritchard, M.T. Role for a Rhammhyaluronan axis in scarless liver repair. Hepatol.,66, 207. **2017**.
14. Rugh, R. The Mouse it's reproduction and development.Burgess Publishing Company. **1976**.
15. Yaping, J. Shenhua, X., XIN, L., Chunwei, L., Gexin, L., Yuanyuan, X. Chunqing, Q. Yuhong, N. and Guifan, S. Arsenic speciation transported through the placenta from mother mice to their newborn pups. *Environmental Research,* **2006**; *101*:349-355.
16. Suckow, M.A; Danneman, P. and Brayton, C. The Laboratory Mouse. 1st ed. CRC Press. **2001**.
17. Al-Aamery, R. A. and Dauod. H. A. A study on amino acid of thyroid gland in two Iraqi vertebrates weasl, Herpestes javanicus (E. Geoffroy Saint. Hilaire, 1818) and long - eared hedgehog Hemiechinus auritus (Gmelina, 1776). *AAJPS.,* **2020**; *1(1),*12-21.
18. Al-Muossawi, B. J. A. Histmorphometric, study on the effect of hydroxychloroquine (HCQ) drug with estimation of amino acid concentration in the kidneys of male albino mice (Mus musculus). (Unpublished MSc thesis). University of Baghdad. **2022**.
19. Al-Hamawandy, D. H. and Al-Bakri, N. A. Analysis Of the Amino Acids. In The Liver Of The Embryo And The Adult Of Domesticated Chicken Gallus Gallus Domesticus (Linnaeus, 1758). *Biochemical and Cellular Archives,* **2019**; *19(2):* 4385-4392.
20. Abed, A. B., Al-Bakri, N.A. and Selman, M.O. Analysis of Amino Acids of the Brain in Embryo and Adult of Quail.IJEIR.,8(1):1-9. **2018**.
21. Lane, M. and Gardner, D. K. Differential regulation of mouse embryo development and viability by amino acids. *Journal of reproduction and fertility*, **1997**; *109(1)*, 153–164.
22. Suenaga R, Tomonaga S, Yamane H et al. Intracerebroventricular injection of L-arginine induces sedative and hypnotic effects under an acute stress in neonatal chicks. *Amino Acids,* **2008**; *35*, 139–146
23. Bear MD, Li M, Liu Y, Giel-Moloney MA, Fanburg BL, and Toksoz D. The Lbc Rho guanine nucleotide exchange factor α-catulin axis functions in serotonin-induced vascular smooth muscle cell mitogenesis and RhoA/ROCK activation. *J. Biol. Chem.*, **2010**; *285(43)*, 32919–32926.
24. Wu, G. Amino acids: metabolism, function and nutrition. *Amino Acids,* **2009**;*37(1),* 1-17.
25. Soghier, L. M. and Brion, L. P. Cysteine, cystine or Nacetylcysteine supplementation in parenterally fed neonates. Cochrane database of systematic. Reviews. Issue 4. No. CD004869. **2006**.
26. Ripps, H. and Shen, W. Review: Taurine: A "very essential" amino acid. Mol. *Vision.,* **2012**; *18*, 2673-2686.
27. Kalinowski, A., Moran, E. T., Jr. and Wyatt, C. Methionine and cystine requirements of slow and fast-feathering male broilers from zero to three weeks of age. Poultry. Sci., **2003**;82, 1423-1427
28. Gstraunthaler G, Holcomb T, Feifel E, Liu W, Spitaler N, and Curthoys NP. Differential expression and acid–base regulation of glutaminase mRNAs in gluconeogenic LLC-PK (1) –FBPase (+) cells. *Am J Physiol Renal Physiol*, **2000**; *278*,227–237.
29. Curthoys NP, and Gstraunthaler G. Mechanism of increased renalgene expression during metabolic acidosis. *Am J Physiol Renal Physiol,* **2001**; *281*: 381–390
30. Cooper AJL, Filc-DeRicco S, and Gelbard AS. L-[13N]-glutamate metabolism in normal rat kidney. In: Bengtson F, Jeppsson B, Almda T, Vilstrup H, eds. Progress in hepatic encephalopathy and metabolic nitrogen exchange (pp.341–51). Boca Raton, FL: CRC Press. **1991**.
31. Van de Poll, M. C., Soeters, P. B., Deutz, N. E., Fearon, K. C., & Dejong, C. H. Renal metabolism of amino acids: its role in interorgan amino acid exchange. *Am. J. clin. nutri.*, **2004**; *79(2)*, 185–197.
32. Badawy, A. A. B. The tryptophan utilization concept in pregnancy. *Obstetrics & Gynecology Science*, **2014**; *57(4)*, 249-259.

33. Newsholme, P., Procopio, J., Lima, M. M., Pithon-Curi, T. C., and Curi, R. Glutamine and glutamate--their central role in cell metabolism and function. *Cell biochemistry and function,* **2003**; *21(1),* 1–9.
34. Parhizkar, S., Latiff, A. L., Rahman, S.A., Dollah, M.A. and Parichehr, H. Assessing estrogenic activity of Nigella sativa in ovariectomized rats using vaginal cornification assay. *AJPP;* **2011**; *5*:137-42
35. Van Winkle, L. J. Amino Acid Transport Regulation and Early Embryo Development. Biol. Reprod., **2001**; 64, 1-12.
36. Xu, W., Zhong, C., Zou, C., Wang, B., and Zhang, N. Analytical methods for amino acid determination in organisms. *Amino Acids*, **2020**; *52(8),* 1071–1088.

Received: May 15, 2023/ Accepted: June 10, 2023 / Published: June 15, 2023
Citation: Abd Ali , A.H.; Al-Bakri , N.A. Estimation of amino acid concentrations in the kidney of albino mice embryo. Revis Bionatura 2023;8 (3) 54. http://dx.doi.org/10.21931/RB/CSS/2023.08.03.54