International Journal of Design & Nature and Ecodynamics
Vol. 19, No. 6, December, 2024, pp. 2041-2047
Journal homepage: http://iieta.org/journals/ijdne

Amino Acid and Hormonal Profiling of the Adrenal Gland in the Pin-Tailed Sandgrouse (*Pterocles alchata*)

Wafaa Barghash Obaid*, Nahla A. Al-Bakri

Department of Biology, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad, Baghdad 10053, Iraq

*Corresponding Author Email: wafaa.obaid2102p@ihcoedu.uobaghdad.edu.iq

https://doi.org/10.18280/ijdne.190620

Received: 27 October 2024
Revised: 6 December 2024
Accepted: 13 December 2024
Available online: 27 December 2024

Keywords:
adrenal gland, amino acid, hormone, Pterocles alchat

ABSTRACT

Amino acids serve structural and functional roles in organisms, while hormones regulate vital physiological processes. The adrenal gland produces life-critical hormones. Profiling amino acids and hormones in the adrenal gland can enhance our understanding of its biochemical composition and regulatory mechanisms. This study investigated the biochemical composition of the adrenal gland in the pin-tailed sandgrouse (*Pterocles alchata*), focusing on amino acid and hormone profiles. Adult sandgrouse were obtained, and their adrenal glands and blood samples were analyzed using high-performance liquid chromatography for amino acids and enzyme-linked immunosorbent assay for hormones. The results identified 18 amino acids in the adrenal gland, with serine (113.29 ± 6.62 µg/mL) and glutamic acid (108.40 ± 4.91 µg/mL) being most abundant, while phenylalanine showed the lowest concentration (37.65 ± 2.70 µg/mL). Hormonal analysis revealed significant seasonal variations. Cortisol, corticosterone, and adrenaline levels were elevated in May compared to February, with May levels recorded at 16.71 ± 0.18, 1.63 ± 0.11, and 41.42 ± 1.88 pg/mL, respectively, versus February levels of 14.63 ± 0.30, 1.14 ± 0.07, and 33.80 ± 1.56 pg/mL. The study revealed adrenal adaptations, key roles of serine and glutamic acid, and seasonal hormonal changes aiding avian conservation insights.

1. INTRODUCTION

Amino acids are organic molecules that contain an amine group (NH_2) and a carboxyl group (COOH), and they are essential components of all forms of life [1, 2]. They are divided into two types: essential amino acids, including valine (Val), leucine (Leu), isoleucine (Ile), phenylalanine (Phe), tryptophan (Trp), methionine (Met), threonine (Thr), histidine (His), lysine (Lys) and arginine (Arg) [3]. The other type of amino acids is non-essential amino acids, which include glycine (Gly), alanine (Ala), proline (Pro), serine (Ser), tyrosine (Tyr), aspartic acid (Asp), and glutamate (Glu). These amino acids can be easily synthesized through intermediate metabolic pathways [4, 5]. Amino acids, like other life molecules, are versatile as they exhibit both structural and functional activities [6, 7]. Many studies have examined the amino acid content in various body organs. Some research has detected the presence of 18 amino acids in the liver of domestic chickens (*Gallus domesticus*) [8], the kidneys of white mice (*Mus musculus*) [9, 10], and the liver of white mice (*Mus musculus*) [11].

On the other hand, the adrenal gland is an important endocrine organ in birds as in mammals [12]. It plays a vital role in several body bioactivities like metabolism, body fluid balance, embryonic development, and immunity [13, 14]. The adrenal cortex produces three types of corticosteroid hormones: mineralocorticoids, glucocorticoids, and androgenic sex hormones. Mineralocorticoid hormones include aldosterone and deoxycorticosterone, which regulate water and electrolyte balance. Glucocorticoid hormones include cortisone, cortisol, and corticosterone, which are crucial for metabolism, stress responses, and anti-inflammatory effects [14, 15]. Studying seasonal changes in adrenal gland hormone levels helps to understand how the organism reacts to the conditions of the surrounding environment [16]. The adrenal glands are essential for maintaining various body functions through hormone production. The adrenal cortex is divided into three distinct zones, each with specific roles. The glomerulosa produces aldosterone, a mineralocorticoid that regulates sodium and potassium balance, blood volume, and blood pressure through the renin-angiotensin-aldosterone system (RAAS) [17]. The second zone is the fasciculata, which secretes cortisol, a glucocorticoid that is vital for glucose metabolism, immune regulation, and the stress response [18]. Reticularis is the third zone responsible for the production of weak androgens, such as dehydroepiandrosterone (DHEA), which contributes to secondary sexual characteristics and serves as a source of estrogen in postmenopausal women [19]. The adrenal medulla generates catecholamines (epinephrine and norepinephrine), which activate the fight-or-flight response, enhancing heart rate, blood pressure, and energy availability during stress [20, 21].

The Iraqi pin-tailed sandgrouse (*Pterocles alchata*), a member of the Pteroclidae family, is characterized by its medium-sized body, long beak, and plumage that closely matches the colors of its natural habitat. The adrenal glands of sandgrouse exhibit distinct adaptations when compared to those of other birds and mammals, reflecting their unique ecological roles and physiological demands [22]. Similar to other avian species, the adrenal glands of sandgrouse have intermingled cortical and medullary tissues, unlike the clearly separated structure found in mammals [23]. Renowned for their remarkable ability to conserve water in arid environments, sandgrouse likely demonstrate enhanced activity in the zona glomerulosa, optimizing aldosterone secretion to efficiently manage water and electrolyte balance [24]. This adaptation is vital for maintaining hydration in desert conditions. In contrast to mammals, the adrenal medulla in sandgrouse is likely specialized for rapid stress responses, though their overall hormonal regulation may differ due to the lack of the mammalian zona reticularis, which produces androgens [25]. These distinctions underscore how adrenal gland structure and function evolve to support the survival strategies of different species [24, 25].

Glands primarily play a crucial role in hormone synthesis and secretion, rather than directly synthesizing amino acids. However, some glands are involved in regulating the balance and utilization of amino acids [26]. Germ cells in the seminiferous tubules of the testis synthesize the amino acid asparagine D-Asp instead of storing it, and its presence in the testicular venous blood plasma of mice supports that the amino acid D-Asp is produced in the seminiferous tubules of the testis and then secreted outside these seminiferous tubules and this acid works to modify the synthesis of the testicular hormone testosterone by Leydig cells, and the amino acid asparagine Asp participates in the synthesis of steroid hormones in mice, and the adrenal gland is one of the organs that stores it instead of synthesizing it, as it is manufactured elsewhere in the body and stored in the adrenal gland after being transported to it by the vascular system [27].

The present study was conducted to investigate the amino acid profile and seasonal fluctuations in hormonal levels of the adrenal gland in the pin-tailed sandgrouse (*Pterocles alchat*), with the goal of understanding the physiological adaptations of this species to its arid and variable environment.

2. MATERIALS AND METHODS

2.1 Source of birds

Adult male *Pterocles alchat* were sourced from a local market (Al-Ghazal market in Baghdad). Their age was around one year and their weight ranged from 190 to 200 g. All birds were housed under standard experimental conditions, including an optimal environmental temperature of 22-24℃, proper ventilation, and unrestricted access to feed and water, in accordance with established animal care protocols. Birds were kept for two weeks to acclimate them to the new environment.

2.2 Sample collection

Three birds were sacrificed and their adrenal glands were extracted for amino acid profiling. For adrenal hormonal analysis, blood samples were collected from 20 birds in February and May, which are considered optimal months for the experiment in Iraq, as these correspond to the spring season. This timing was chosen to minimize the effects of extreme seasonal variations on the hormonal levels of the birds, ensuring more consistent and reliable results. The bird wings were sterilized and blood was collected with a 3 ml syringe. Venipuncture, the method used for blood collection, commonly targets the jugular vein, cutaneous ulnar vein (wing vein), and medial metatarsal vein, with these sites being preferred for their accessibility and blood yield. Venipuncture is considered the most reliable technique for obtaining diagnostically accurate samples, as it provides core blood rather than peripheral capillary blood, which can interfere with hormone analysis results. Among these sites, the jugular vein is often the first choice, especially in smaller birds, due to its larger size and higher blood yield, as noted by several avian research studies [28]. The blood-containing vials were centrifuged to separate the serum, which was kept in the Eppendorf tubes in the freezer (-20℃) until they were used for analysis [29].

2.3 Amino acid analysis

The high-performance liquid chromatography (HPLC) method was employed to quantify the amino acids. This analysis was conducted in the laboratory of the Department of Material Research, Ministry of Science and Technology, utilizing reversed-phase C18 silica-based columns. The system used for the separation and detection of amino acids was the Agilent 1260 model from the USA, which is known for its precision and reliability in HPLC analysis. The procedure was conducted following the method described by Fierabracci et al. [30]. One gram of the adrenal gland of the pin-tailed sandgrouse was homogenized manually in 20 ml of deionized water and then 1.5 g of sulfosalicylic acid was added. The samples were centrifuged for one hour, after which concentrated hydrochloric acid (0.1N, pH 2.0) was added to the supernatant. The mixture was then centrifuged at 3,000 rpm to further concentrate and prepare the sample for analysis. Ten microliters (10 µL) of the clear solution were mixed with 10 µL of phenyl isothiocyanate (PITC). After allowing the reaction to proceed for one minute, 50 µL of sodium acetate (0.1M, pH 7.0) was added to the mixture to stabilize the reaction and facilitate the derivatization process. Finally, the amino acid concentrations were measured using HPLC method. The amino acid concentration was calculated using the following formula:

$$\begin{aligned} &Amino\ acid\ concentration \\ &= \frac{Sample\ band\ area}{stanadrd\ band\ area}\ Std\ Conc. \\ &\times\ Dilution\ Numbers \end{aligned}$$

2.4 Estimation of adrenal hormones

The enzyme-linked immunosorbent assay (ELISA) kit (Sunlong Biotech) was used to measure adrenal hormones following the manufacturer’s instructions.

3. RESULTS

3.1 Amino acid content of adrenal gland in *Pterocles alchata*

Amino acid analysis of the adrenal gland in the pin-tailed sandgrouse, conducted using HPLC, revealed the presence of

18 amino acids, with significant ($p \leq 0.001$) differences between their concentrations. Serine (Ser) exhibited the highest concentration, while phenylalanine (Phe) recorded the lowest concentration in the adrenal gland amino acid profile, as shown in Table 1. Among the amino acids, serine (Ser) had the highest concentration at 113.290 ± 6.62 µg/mL, followed by glutamic acid at 108.395 ± 4.91 µg/mL, alanine (Ala) at 90.721 ± 3.45 µg/mL, and asparagine (Asp) at 88.901 ± 3.60 µg/mL. Other notable concentrations included histidine (His) at 61.237 ± 2.00 µg/mL, glycine (Gly) at 59.028 ± 9.07 µg/mL, glutamine (Glu) at 59.016 ± 2.88 µg/mL, and proline (Pro) at 57.211 ± 8.56 µg/mL. Threonine (Thr) and citrulline (Cit) recorded concentrations of 40.566 ± 2.29 µg/mL and 47.817 ± 1.85 µg/mL, respectively, while arginine (Arg) and tyrosine (Tyr) were measured at 45.015 ± 4.99 µg/mL and 60.336 ± 4.16 µg/mL, respectively. The group of branched-chain amino acids (BCAAs) was also observed, including valine (Val), leucine (Leu), and isoleucine (Ile), with concentrations of 52.234 ± 6.12 µg/mL, 57.483 ± 4.77 µg/mL, and 78.279 ± 2.35 µg/mL, respectively. Methionine (Met) was recorded at 68.463 ± 5.36 µg/mL, phenylalanine (Phe) at 37.654 ± 2.70 µg/mL, and lysine (Lys) at 63.449 ± 3.21 µg/mL (Table 1 and Figure 1).

Table 1. Amino acid concentrations of the adrenal gland in *Pterocles alchat*

Amino Acid	Conc. of Amino Acid (µg/mL)
Asparagine (Asp)	88.901±3.60
Serine (Ser)	113.290±6.62
Glutamine (Glu)	59.016±2.88
Glutamic acid	108.395±4.91
Threonine (Thr)	40.566±2.29
Histidine (His)	61.237±2.0
Citrulline (Cit)	47.817±1.85
Alanine (Ala)	90.721±3.45
Proline (Pro)	57.211±8.56
Glycine (Gly)	59.028±9.07
Arginine (Arg)	45.015±4.99
Tyrosine (Tyr)	60.336±4.16
Valine (Val)	52.234±6.12
Methionine (Met)	68.463±5.36
Isoleucine (Ile)	78.279±2.35
Leucine (Leu)	57.483±4.77
Phenylalanine (Phe)	37.654±2.70
Lysine (Lys)	63.449±3.21

Data were expressed as mean ± standard error.

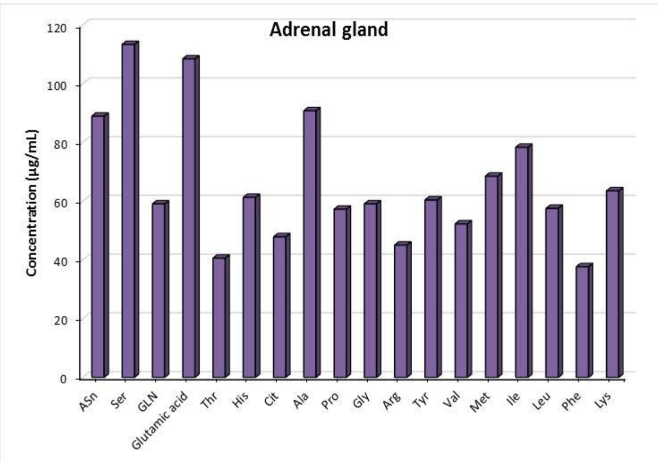

Figure 1. Amino acid concentrations of the adrenal gland in *Pterocles alchat* bird

3.2 Estimation of adrenal hormones

As shown in Table 2 and Figure 2, the concentrations of adrenaline hormone were significantly higher in May compared to February. In February, the concentrations of cortisol, corticosterone, and adrenaline were 14.629 ± 0.299, 1.141 ± 0.073, and 33.796 ± 1.559 pg/mL, respectively. In May, these concentrations increased to 16.714 ± 0.181, 1.631 ± 0.106, and 41.422 ± 1.879 pg/mL, respectively.

Table 2. Hormone levels in the adrenal gland of *Pterocles alchat* during February and May

Parameter	Group	Mean±SE (pg/mL)	P value
Cortisol	February	14.629±0.299	<0.0001**
	May	16.714±0.181	
Corticosterone	February	1.141±0.073	<0.0001**
	May	1.631±0.106	
Adrenaline	February	33.796±1.559	0.002**
	May	41.422±1.879	

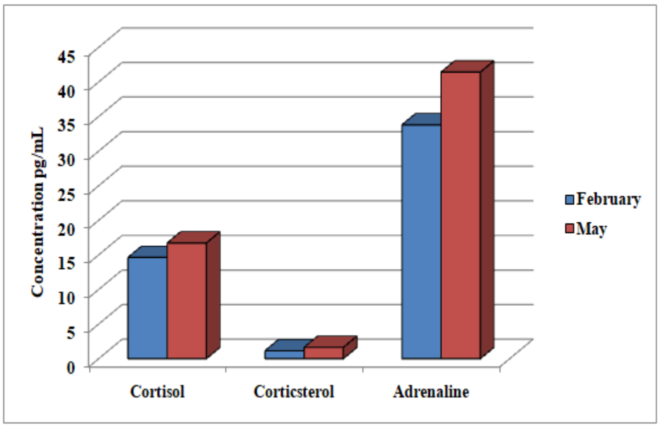

Figure 2. Hormones concentrations of the adrenal glandin *Pterocles alchat* bird in February and May

4. DISCUSSION

The results of the present study identified the presence of 16 amino acids in the adrenal gland of the pin-tailed sandgrouse. The amino acids have different structural and functional activities. The adrenal gland responds to chronic stress by producing various substances, including cytokines, prostaglandins, and nitric oxide (NO). Arginine serves as the primary substrate for the synthesis of nitric oxide, a process facilitated by the enzyme nitric oxide synthase [31]. Nitric oxide contributes to many important functions in the body, such as regulating blood pressure, adrenal gland function, synthesis of steroid hormones, neurotransmission, stimulation of the secretion of the stress hormone corticosterone, and strengthening the body's defenses mechanism [32]. Numerous studies have demonstrated that arginine supplementation offers several beneficial effects on glandular function. Arginine stimulates the release of pancreatic insulin, growth hormone, glucagon, and pituitary prolactin [33-35]. Mäkinen et al. [36] reported the presence of 9–18 amino acids, including arginine, tryptophan, and proline, in varying proportions, along with fixed levels of leucine and isoleucine, in saliva collected from the palatine glands of young adults aged 22 years. Similarly, Kiriyama and Nochi [37] identified serine as a prominent amino acid in endocrine organs such as the adrenal gland, pituitary, pancreas, and testes in mice. Furthermore, the amino acid asparagine (Asn), synthesized from aspartate and glutamine [38], is found in significant quantities in the adrenal and pituitary glands, where it plays a crucial role in various vital functions in mammals. It stimulates the secretion of the melatonin hormone from the pineal gland and increases the production of testosterone hormone from the Lydig cells. Also, it stimulates the secretion of the prolactin, growth, and luteinizing hormones from the anterior lobe of the pituitary gland in mice [37].

Various studies have shown that asparagine (Asn) levels in endocrine glands such as the pituitary, pineal, adrenal glands, and testes increase significantly during development and postpartum, eventually stabilizing once the glands are fully differentiated. This suggests that asparagine plays a crucial role in the maturation of these glands [39]. Studies on changes in asparagine (Asn) levels during the formation of adrenal tissue in mice postpartum revealed a significant increase in asparagine (Asn) concentration, peaking at 608 ± 70 nmol/g during the third week of life. By the fourteenth week, the concentration decreased to 28 ± 2 nmol/g [40, 41]. Lee et al. [42] reported that the asparagine (Asn) content in the tissues changes significantly during the development of mammalian embryos. A significant increase in asparagine (Asn) levels was observed during the development of the human brain, mouse brain, pineal gland, adrenal gland, pituitary gland, and testes in mice, as well as during brain development and growth in chicken embryos [43]. Additionally, Kuikarni et al. [44] reported that glutamine, one of the most abundant amino acids in the body, is synthesized from glutamic acid and ammonia. This amino acid plays a crucial role in the function of certain endocrine glands, including the prostate gland.

The findings of the present study showed that there are statistically significant differences in the concentration of adrenal hormones. A significant difference in cortisol concentration was observed, with levels reaching 14.629 in February and 16.714 in May. This finding aligns with the study by Sarma et al. [45], which investigated the effect of different seasons on cortisol levels in the serum of broiler chickens. They found that cortisol concentrations were higher in adult chickens at 42 days of age during hot seasons, likely due to elevated environmental temperatures and the resulting heat stress. Sohail et al. [46] also reported that heat stress significantly increases the concentration of cortisol, during hot seasons. The body requires more energy to mitigate the heat stress effects. This process involves the production of additional glucose from non-carbohydrate sources through the catabolism of muscle protein. The hypothalamus activates and releases adrenocorticotropic hormone (ACTH), which stimulates the adrenal cortex to release cortisol into the blood. The findings also align with the study by Majekodunmi et al. [47], which found that exposure to high temperatures resulted in elevated cortisol concentrations in broiler chickens. A major environmental condition that harms various birds is heat stress. Heat exposure has been linked to changes in the chickens' metabolic and immune systems, as well as an increase in the number of deaths [48, 49]. The findings of the current study revealed a significant increase in corticosterone concentration in the Iraqi pin-tailed sandgrouse during May, when temperatures ranged between 37 and 39℃, with a concentration of 1.631 ± 0.106.

Seasonal hormone fluctuations in birds are influenced by environmental cues like photoperiod, temperature, and food availability. These factors regulate the HPA and HPG axes, modulating hormones for survival and reproduction. Increased daylight boosts glucocorticoid and adrenaline levels, supporting breeding and migration, while food availability affects reproductive hormones [50]. Cold seasons reduce corticosterone to conserve energy, whereas warmer periods enhance hormonal activity. Melatonin, governed by photoperiod, aligns physiological processes with seasons, ensuring homeostasis and reproductive success across varying environments, aiding ecological and conservation research [51].

Sudhakumari et al. [52] reported in studying the activity of adrenal glands and gonads in the Indian tropical forest quail Perdiculaasictica that increasing day length (photoperiod), temperature and rainfall were positively correlated with adrenal gland and gonad activity, as a significant increase in the weights of adrenal glands was observed during the active reproductive phase of both males and females and decreased during the inactive reproductive phase.

Tropical changes in environmental factors can be very different. For example, there are large changes in humidity, rainfall and temperature in tropical regions. These can be considered environmental stressors that affect the activity of the adrenal gland. High temperatures during the summer months may contribute to an increase in the function of the adrenal gland. Rainfall can also be an obstacle to the migration activity of many migratory birds. However, the adrenal gland maintains high activity during these stages. The adrenal gland also plays an important role in regulating the internal environment of bird’s bodies, as its hormones affect growth, tissue differentiation, metabolism regulation and the bird’s body’s resistance to disease, poisoning, stress and low temperature. These results indicate that changes in external environmental conditions such as changes in temperature and rainfall affect the activity of the adrenal gland through an increase or decrease in the secretion of hormones [53, 54].

Studies on Japanese quail, pigeons, chickens, turkeys and ducks have shown that corticosteroid concentrations in the blood vary between day and night, with high concentrations being obtained at night. It is believed that there are differences in the concentration of this hormone with the circadian rhythm, as it provides energy to endothermic animals during the night when they feed or move. For nocturnal species such as owls, its concentrations may be more closely related to the activity period than to the light-dark cycle [55].

5. CONCLUSIONS

This study provides valuable insights into the amino acid and hormonal profiles of the adrenal glands in the Iraqi pin-tailed sandgrouse (*Pterocles alchata*). The findings revealed the presence of 18 amino acids, with serine exhibiting the highest concentration, highlighting its role in the adrenal function. Additionally, significant seasonal fluctuations in adrenal hormones, particularly corticosterone and cortisol, were observed, with elevated levels in May corresponding to warmer temperatures. These findings underscore the sandgrouse's physiological adaptation to its arid environment, where hormonal regulation plays a crucial role in managing stress and maintaining metabolic homeostasis. The study offers a deeper understanding of the species' survival strategies in response to environmental stressors, which could inform conservation efforts and further research into avian endocrinology.

ACKNOWLEDGMENT

The authors thank the scientists in the Department of Biology at the College of Education for Pure Science (Ibn-Al-Haitham), University of Baghdad, for their invaluable assistance towards conducting this research.

REFERENCES

[1] Nelson, D.L., Cox, M.M. (2017). Lehninger Principles of Biochemistry (7th ed.). W. H. Freeman.

[2] Greco, E, Winquist, A., Lee T.J., Collins, S., Lebovic, Z., Zerbe-Kessinger, T., Mihan, A.J. (2017). The role of source of protein in regulation of food intake, satiety, body weight and body composition. Journal of Nutritional Health & Food Engineering, 6(6): 186-193. https://doi.org/10.15406/jnhfe.2017.06.00223

[3] Salman, A.D. (2014). The Protective role of Pomegranate seed oil (Pometon) on kidney (functional and structural) damage induced by Methionine overload in adult female Rabbits. The Iraqi Journal of Veterinary Medicine, 38(2): 66-73.

[4] Akram, M., Asif, H.M., Uzair, M., Akhtar, N., Madni, A., Shah, S.A., ul Hasan, Z., Ullah, A. (2011). Amino acids: A review article. Journal of Medicinal Plants Research, 5(17): 3997-4000.

[5] Qaid, M.M., Al-Garadi, M.A. (2021). Protein and amino acid metabolism in poultry during and after heat stress: A review. Animals, 11(4): 1167. https://doi.org/10.3390/ani11041167

[6] Verrey, F., Singer, D., Ramadan, T., Vuille-dit-Bille, R.N., Mariotta, L., Camargo, S.M. (2009). Kidney amino acid transport. Pflügers Archiv-European Journal of Physiology, 458: 53-60. https://doi.org/10.1007/s00424-009-0638-2

[7] AL-Imam, M.J., AL-Rubaii, B.A.L. (2016). The influence of some amino acids, vitamins and anti-inflammatory drugs on activity of chondroitinase produced by Proteus vulgaris caused urinary tract infection. Iraqi Journal of Science, 57(4A): 2412-2421.

[8] Al-Hamawandy, D.H., Al-Bakri, N.A. (2019). Analysis of the amino acids in the liver of the embryo and the adult of domesticated chicken Gallus gallus domesticus (Linnaeus, 1758). Biochemical & Cellular Archives, 19(2): 4385-4392. https://doi.org/10.35124/bca.2019.19.2.4385

[9] Al-Musawiu, B.J., Al-Bakri, N.A. (2022). Effect of Hydroxychloroquine Drugs on Amino Acids in the Kidneys of White Mice (Mus muscules). International Journal of Drug Delivery Technology, 12(4): 1590-1594.

[10] Ali, A.H., Al-Bakri, N.A. (2023). Estimation of amino acid concentration in the kidney of albino mice embryo. Latin American Journal of Biotechnology and Life Sciences, 8(3): 1-9. https://doi.org/10.21931/RB/CSS/2023.08.03.54

[11] Ahmad, T.M., Al-Bakri, N. (2022). Effect of zinc (Zn) drug on amino acid concentration in the liver of white mice, Mus musculus. International Journal of Drug Delivery Technology, 12(4): 1838-1843.

[12] Ahmed, N.A., Dauod, H.A.M. (2016). Morphological description and histological structure of adrenal gland in black Iraqi partridge *Francolinus francolinus*. Iraqi Journal of Science, 57(1B): 330-337.

[13] Batah, A.L., Mirhish, S.M. (2019). Histomorphological and histochemical study of adrenal gland in adult male of guinea pigs (Cavia porcellus). The Iraqi Journal of Veterinary Medicine, 43(1): 59-66.

[14] Kadhim, A.B., Khaleel, I.M. (2021). Comparison of histomorphometric study of chromaffin cells in adult male squirrel (*Sciurus anomalus*) and hamster (*Mesocricetus auratus*). The Iraqi Journal Veterinary Medicine, 45(1): 46-50.

[15] Alwan, A.F. (2014). Fetal adrenal cortex development with maternal serum cortisol profiles in relation to estrous, laparoscopic insemination in Iraqi goats out the breeding season. The Iraqi Journal of Veterinary Medicine, 38(1): 129-136.

[16] Schradin, C. (2008). Seasonal changes in testosterone and corticosterone levels in four social classes of a desert-dwelling sociable rodent. Hormones and Behavior, 53(4): 573-579. https://doi.org/10.1016/j.yhbeh.2008.01.003

[17] Gambaryan, S., Mohagaonkar, S., Nikolaev, V.O. (2023). Regulation of the renin-angiotensin-aldosterone system by cyclic nucleotides and phosphodiesterases. Frontiers in Endocrinology, 14: 1239492. https://doi.org/10.3389/fendo.2023.1239492

[18] Huber, H.F., Li, C., Xie, D., Gerow, K.G., Register, T.C., Shively, C.A., Cox, L.A., Nathanielsz, P.W. (2024). Female baboon adrenal zona fasciculata and zona reticularis regulatory and functional proteins decrease across the life course. GeroScience, 46(3): 3405-3417. https://doi.org/10.1007/s11357-024-01080-9

[19] Dumontet, T., Martinez, A. (2021). Adrenal androgens, adrenarche, and zona reticularis: A human affair? Molecular and Cellular Endocrinology, 528: 111239. https://doi.org/10.1016/j.mce.2021.111239

[20] William Tank, A., Lee Wong, D. (2011). Peripheral and central effects of circulating catecholamines. Comprehensive Physiology, 5(1): 1-15. https://doi.org/10.1002/cphy.c140007

[21] Lopez Ruiz, J.R., Ernst, S.A., Holz, R.W., Stuenkel, E.L. (2022). Basal and stress-induced network activity in the adrenal medulla in vivo. Frontiers in Endocrinology, 13: 875865. https://doi.org/10.3389/fendo.2022.875865

[22] de Matos, R. (2008). Adrenal steroid metabolism in birds: Anatomy, physiology, and clinical considerations. Veterinary Clinics of North America: Exotic Animal Practice, 11(1): 35-57. https://doi.org/10.1016/j.cvex.2007.09.006

[23] Bechmann, N., Berger, I., Bornstein, S.R., Steenblock, C. (2021). Adrenal medulla development and medullary-cortical interactions. Molecular and Cellular Endocrinology, 528: 111258. https://doi.org/10.1016/j.mce.2021.111258

[24] Hill, P.A., Coghlan, J.P., Butkus, A., Ryan, G.B. (1983). Structural and functional studies of the adrenal zona glomerulosa in sodium-depleted and sodium-loaded sheep. Cell and Tissue Research, 229: 515-531. https://doi.org/10.1007/BF00207695

[25] Goldstein, D.S. (2010). Adrenal responses to stress. Cellular and Molecular Neurobiology, 30: 1433-1440. ttps://doi.org/10.1007/s10571-010-9606-9

[26] Nagata, Y., Homma, H., Lee, J.A., Imai, K. (1999). D-Aspartate stimulation of testosterone synthesis in rat Leydig cells. FEBS Letters, 444(2-3): 160-164. https://doi.org/10.1016/S0014-5793(99)00045-9

[27] Gjerstad, J.K., Lightman, S.L., Spiga, F. (2018). Role of glucocorticoid negative feedback in the regulation of HPA axis pulsatility. Stress, 21(5): 403-416. https://doi.org/10.1080/10253890.2018.1470238
[28] Lumeij, J.T. (2011). Avian clinical pathology. General considerations. Veterinary Quarterly, 9(3): 249-254. https://doi.org/10.1080/01652176.1987.9694109
[29] Younis, M.E., Jaber, F.A., Majrashi, K.A., Ghoneim, H.A., Shukry, M., Shafi, M.E., Albaqami, N.M., El-Hack, M.E.A., Ghanima, M.M.A. (2023). Impacts of synthetic androgen and estrogenic antagonist administration on growth performance, sex steroids hormones, and immune markers of male and female broilers. Poultry Science, 102(1): 102244. https://doi.org/10.1016/j.psj.2022.102244
[30] Fierabracci, V., Masiello, P., Novelli, M., Bergamini, E. (1991). Application of amino acid analysis by high-performance liquid chromatography with phenyl isothiocyanate derivatization to the rapid determination of free amino acids in biological samples. Journal of Chromatography B: Biomedical Sciences and Applications, 570(2): 285-291. https://doi.org/10.1016/0378-4347(91)80531-G
[31] Kleinert, H., Schware, P.M., Forstreman, U. (2003). Regulation of the expression of inducible nitric oxide synthase. Biological Chemistry, 384(10-11): 1343-1364.
[32] Cymeryng, C.B., Dada, L.A., Colonna, C., Menolez, C.P., Podesta, E.J. (1999). Effects of L-arginine in rat adrenal cells: Involvement of nitric oxide synthase. Endocrinology, 140(7): 2962-2967. https://doi.org/10.1210/endo.140.7.6848
[33] Böger, R.H., Bode-Böger, S.M., Mügge, A., Kienke, S., Brandes, R., Dwenger, A., Frölich, J.C. (1995). Supplementation of hypercholesterolaemic rabbits with L-arginine reduces the vascular release of superoxide anions and restores NO production. Atherosclerosis, 117(2): 273-284. https://doi.org/10.1016/0021-9150(95)05582-H
[34] Lubec, B., Hayn, M., Kitzmuller, E. (1997). L-Arginine reduces lipid peroxidation in patients with diabetes mellitus. Free Radical Biology and Medicine, 22(1): 355-357. https://doi.org/10.1016/S0891-5849(96)00386-3
[35] Abdel Malak, H.W., Amin, M.A. (2018). Protective role of L-arginine on the suprarenal gland of adult male albino rats subjected to recurrent acute restraint stress: A histological and immunohistochemical study. The Egyptian Journal of Anatomy, 41(1): 156-171.
[36] Mäkinen, K.K., Virtanen, K.K., Mäkinen, P.L., Kotiranta, J. (1988). Free amino acids in human palatine gland secretions. Archives of Oral Biology, 33(11): 847-849. https://doi.org/10.1016/0003-9969(88)90111-2
[37] Kiriyama, Y., Nochi, H. (2016). D‐amino acids in the nervous and endocrine systems. Scientifica, 2016(1): 6494621. https://doi.org/10.1155/2016/6494621
[38] He, W., Li, P., Wu, G. (2021). Amino acid nutrition and metabolism in chickens. In: Amino Acids in Nutrition and Health, pp. 109-131. https://doi.org/10.1007/978-3-030-54462-1_7
[39] Usiello, A., Di Fiore, M.M., De Rosa, A., Falvo, S., Errico, F., Santillo, A., Nuzzo, T., Chieffi Baccari, G. (2020). New evidence on the role of d-aspartate metabolism in regulating brain and endocrine system physiology: From preclinical observations to clinical applications. International Journal of Molecular Sciences, 21(22): 8718. https://doi.org/10.3390/ijms21228718
[40] Hashimoto, A., Oka, T., Nishikawa, T. (1995). Anatomical distribution and postnatal changes in endogenous free D‐aspartate and D‐serine in rat brain and periphery. European Journal of Neuroscience, 7(8): 1657-1663. https://doi.org/10.1111/j.1460-9568.1995.tb00687.x
[41] Sakai, K., Homma, H., Lee, J.A., Fukushima, T., Santa, T., Tashiro, K., Iwatsubo, T., Imai, K. (1997). D-Aspartic acid localization during postnatal development of rat adrenal gland. Biochemical and Biophysical Research Communications, 235(2): 433-436. https://doi.org/10.1006/bbrc.1997.6783
[42] Lee, J.A., Long, Z., Nimura, N., Iwatsubo, T., Imai, K., Homma, H. (2001). Localization, transport, and uptake of D-aspartate in the rat adrenal and pituitary glands. Archives of Biochemistry and Biophysics, 385(2): 242-249. https://doi.org/10.1006/abbi.2000.2163
[43] Homma, H. (2002). D-Aspartate in the mammalian body. Viva Origino, 30(4): 204-215 https://doi.org/10.50968/vivaorigino.30.4_204
[44] Kulkarni, C., Kulkarni, K.S., Hamsa, B.R. (2005). L-Glutamic acid and glutamine: Exciting molecules of clinical interest. Indian Journal of Pharmacology, 37(3): 148-154. https://doi.org/10.4103/0253-7613.16210
[45] Sarma, M., Deka, D., Deka, A., Kalita, K.P., Sapcota, D., Mahanta, J.D., Bhattacharyya, B.N., Deori, S. (2022). Study of impact of different seasons on HPS-70 and cortisol level of broiler chicken in different agro-climatic zones of Assam. The Pharma Innovation Journal, 11(8S): 1374-1378.
[46] Sohail, M.U., Ijaz, A., Yousaf, M.S., Ashraf, K., Zaneb, H., Aleem, M., Rehman, H. (2010). Alleviation of cyclic heat stress in broilers by dietary supplementation of mannan-oligosaccharide and Lactobacillus-based probiotic: Dynamics of cortisol, thyroid hormones, cholesterol, C-reactive protein, and humoral immunity. Poultry Science, 89(9): 1934-1938. https://doi.org/10.3382/ps.2010-00751
[47] Majekodunmi, B.C., Ogunwole, O.A., Sokunbi, O.A. (2016). Plasma corticosterone and adrenal gland histomorphometry of heat stressed broiler chickens given supplemental electrolytes or vitamin C. Archivos de Zootecnia, 65(252): 535-540.
[48] Nawab, A., Ibtisham, F., Li, G., Kieser, B., Wu, J., et al. (2018). Heat stress in poultry production: Mitigation strategies to overcome the future challenges facing the global poultry industry. Journal of Thermal Biology, 78: 131-139. https://doi.org/10.1016/j.jtherbio.2018.08.010
[49] Sesay, A.R. (2022). Impact of heat stress on chicken performance, welfare, and probable mitigation strategies. International Journal of Environment and Climate Change, 12(11): 3120-3133. https://doi.org/10.9734/ijecc/2022/v12i111360
[50] Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M., Richardson, R.D. (1998). Ecological bases of hormone—behavior interactions: The “emergency life history stage”. American Zoologist, 38(1): 191-206. https://doi.org/10.1093/icb/38.1.191
[51] Dawson, A., King, V.M., Bentley, G.E., Ball, G.F. (2001). Photoperiodic control of seasonality in birds. Journal of Biological Rhythms, 16(4): 365-380.

https://doi.org/10.1177/074873001129002079
[52] Sudhakumari, C.C., Haldar, C., Senthilkumaran, B. (2001). Seasonal changes in adrenal and gonadal activity in the quail, Perdicula asiatica: Involvement of the pineal gland. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 128(4): 793-804. https://doi.org/10.1016/S1096-4959(01)00302-5
[53] Yiallouris, A., Filippou, C., Themistocleous, S.C., Menelaou, K., Kalodimou, V., Michaeloudes, C., Johnson, E.O. (2024). Aging of the adrenal gland and its impact on the stress response. Vitamins and Hormones, 124: 341-366. https://doi.org/10.1016/bs.vh.2023.12.004
[54] Montaruli, A., Castelli, L., Mulè, A., Scurati, R., Esposito, F., Galasso, L., Roveda, E. (2021). Biological rhythm and chronotype: New perspectives in health. Biomolecules, 11(4): 487. https://doi.org/10.3390/biom11040487
[55] Westerhof, I. (1998). Pituitary function and glucorticoid administration in Pigeons (Columba liviadomestica). Journal of Avian Medicine and Surgery, 12(3): 167-177. https://www.jstor.org/stable/30133169.