

Effects of Dietary L-Tyrosine on Dopaminergic Neurons and Cutaneous Melanosomes in the Silky Fowl

Efectos de la L-Tirosina Dietética Sobre las Neuronas Dopaminérgicas y los Melanosomas Cutáneos en la Gallina Sedosa

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SUMMARY: The silky fowl is characterized by hyperpigmentation. Currently, however, the physiological mechanism of hyperpigmentation has not been fully disclosed. Additionally, peripheral melanocytes and central dopaminergic neurons produce melanin and neuromelanin by the same substrate – L-tyrosine, respectively, but the relationship between dopaminergic neurons and peripheral melanogenesis remains unclear. In the present study, different levels of dietary L-tyrosine are fed to different groups of the silky fowl. After 4 weeks, midbrain dopaminergic neurons and melanosomes of cutaneous melanocytes are identified and counted by paraffin section, tyrosine hydroxylase (TH) immunohistochemistry and transmission electron microscopy, respectively. Within 0-0.8 % concentration range, L-tyrosine enhances the number of dopaminergic neurons and melanosomes ($P < 0.05$). Moreover, the increased number of dopaminergic neurons and that of melanosomes is positively correlated ($R^2 = 0.6279$). The results demonstrate that L-tyrosine promotes the increase of midbrain dopaminergic neurons and melanosomes of cutaneous melanocytes. L-tyrosine regulates positively cutaneous melanogenesis through dopaminergic neurons via the TH in silky fowl.

KEY WORDS: Chicken; Dopaminergic cell; Tyrosine hydroxylase; Melanosome.

INTRODUCTION

The silky fowl (*Gallus gallus domesticus* Brisson) is an economically important avian species in China and Southeast Asia. The meat and eggs of silky fowl have high antioxidant activity, nutritional and medicinal value (Zhang *et al.*, 2022; Liao *et al.*, 2022; Qiu *et al.*, 2023; Ju *et al.*, 2025). Phenotypically, a unique feature of the silky fowl is the presence of numerous melanocytes/melanin in various organs except feather. Because of physiological hyperpigmentation, the silky fowl is considered an excellent animal model for studying the mechanism of melanogenesis (Dorshorst *et al.*, 2010). In addition, melanin plays many important roles, such as radioprotection, antioxidation, predator-prey interactions, thermoregulation, disease resistance, inhibiting virus, and inhibiting mtDNA damage and mitochondrial overoxidation in the body (ElObeid *et al.*, 2017; Liao *et al.*, 2022; Qiu *et al.*, 2023; Liu *et al.*, 2025). As a result, the presence of melanin may be the cause of its medicinal value and therapeutic effect. Thus, melanin is an economically important trait that determines the first

impression of a consumer toward a broiler and can ultimately affect consumer choice in the market and economic value of the silky fowl. Currently, the genetic basis, influencing factors, molecular mechanisms of melanogenesis are gradually being elucidated in the silky fowl (Huang *et al.*, 2023). However, the information on physiological and cytological mechanisms involved in hyperpigmentation is still very limited.

Melanin is most often produced in melanocytes, which are mainly located in the basal layer of the epidermis (Bonaventure *et al.*, 2013). However, another melanin – neuromelanin is produced by oxidation of catecholamines in the central nervous system (Hatch *et al.*, 2025). Previous studies have confirmed that dopaminergic neurons have been shown to be involved in neuromelanin biosynthesis (Liang *et al.*, 2004; Nagatsu *et al.*, 2023). Currently, data on the relationships between central dopaminergic neurons and peripheral melanogenesis remains scarce. The present study

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sought to determine the relationships between midbrain dopaminergic neurons and cutaneous melanocytic melanosomes in the silky fowl, and attempt to understand the cytologic mechanism of physiological hyperpigmentation in avian. Elucidation of peripheral melanogenesis mechanisms will contribute to the regulation of neuromelanogenesis. Therefore, it is of great significance for screening candidates for inhibition of central neuromelanogenesis and the treatment of Parkinson's disease in human in future.

MATERIAL AND METHOD

Animals and experimental design

The 180 one-day-old silky fowls are randomly divided into 6 groups (Three repeats per group), and the 30 chickens of each group are fed by the diet (Table I) with different levels of L-tyrosine, control, 0.2 %, 0.4 %, 0.6 %, 0.8 % and 1.0 %, respectively. After 4 weeks, all chickens are executed after anesthesia by ethyl ether, and the tissues are excised. All animal procedures were approved by the animal ethical committee of Foshan University (Foshan, China; No. Fosu2022038).

Tyrosine hydroxylase (TH) immunohistochemistry (IHC)

TH IHC is employed for midbrain dopaminergic neurons identification. The midbrains of the chickens are sampled and fixed in 4 % paraformaldehyde/PBS, and then are made into paraffin sections (5 μ m). After deparaffination, the sections are pre-treated by heat mediated antigen retrieval in 0.01 M sodium citrate buffer (pH 6.0) at 95 °C for 15 min. After cooling, the sections are incubated in 3 % H₂O₂ and goat serum for 15 min each, and then they are incubated in the anti- TH antibodies (1:200 dilution) (Millipore, USA) at 37 °C for 3 h. The sections are incubated with anti-mouse IgG

and horseradish peroxidase labeled streptomyces ovalbumin for 15 min each. Afterwards, the sections are visualized with 3,3-diaminobenzidine (DAB) chromogenic kit. After dehydrating and mounting, the microphotographs are taken by the BM 2000 light biological microscopy (Nanjing, China).

Transmission electron microscopy (TEM)

The ultrastructure of cutaneous melanocytes and their melanosomes is identified by TEM. Briefly, small pieces of dorsal skin are excised and fixed in 2.5 % glutaraldehyde/PBS for 24 h at 4 °C and post-fixed in 1 % OsO₄ for 2 h. The samples are dehydrated in ethanol and acetone, and then embedded in Epon812. The samples are sectioned with an LKB-V type ultramicrotome (Stockholm, Sweden), and stained with 1 % uranyl acetate and Reynold's lead citrate for 20 min. Finally, the stained sections are observed and photographed by a Hitachi H-600 TEM with a high-resolution digital camera (Tokyo, Japan).

Statistical analysis

The quantitative analysis for TH immunopositive cells and melanocytic melanosomes of skin is performed, respectively. Data are analyzed by Office Excel (Microsoft, USA) and SPSS 19.0 software (IBM, USA). The number of IH immunopositive cells and melanosomes is showed as mean $\pm$ standard error ($\bar{x} \pm s$), histograms and line charts, respectively. The differences between groups are tested by Least Significant Difference (LSD). Moreover, the correlation is analyzed between the increased number of dopaminergic neurons and that of melanosomes.

RESULTS

The effects of L-tyrosine on the number of TH immunopositive cells

The TH immunopositive cells are observed in all groups (Fig. 1a-1f). Their cell bodies appear as subrotund, cone-shaped and polygon. The cytoplasmic processes are frequently observed. The cytoplasm and processes of TH immunopositive cells show brown, but their nuclei are negative, and show pale and vesicular. From the control group to the 0.8 % group, the color of the cytoplasm is gradually deepened, and immunopositive is gradually strengthened. In particular, in the 0.6 % and 0.8 % group, TH immunopositive cells show stronger immunostain cytoplasm and processes, and the color approximates to chocolate (Fig. 1d, 1e). However, in the 1.0 %

Table I. Composition and nutrient levels of basal diet.

Ingredient	Content, %	Nutrient composition	Content, %
Com	51.40	Metabolizable energy, MJ/kg	12.16 ²⁾
Com starch	13.50	Crude protein ²⁾	17.41
Peanut meal	18.60	Ca ²⁾	0.97
Wheat bran	3.00	Available phosphorus ²⁾	0.46
Rapeseed meal	7.00	Met ³⁾	0.35
Rapeseed oil	2.00	Lys ³⁾	0.94
NaCl	0.30	Tyr ³⁾	0.38
Limestone	1.30		
CaHPO ₄	1.70		
Premix ¹⁾	0.60		

1) Premix provided per kg of diet: VA 12 500 IU; VD3 3 750 IU; VE 20 IU; VK3 3.75 mg; VB1 3.75 mg; VB2 8 mg; VB6 3.75 mg; VB12 25 mg; biotin 100 mg; nicotinic acid 50 mg; folic acid 1.25 mg; calcium pantothenate 12.5 mg; Cu 8 mg; Fe 80 mg; Zn 40 mg; Mn 60 mg; I 0.35 mg; Se 0.15 mg. 2) Calculated values. 3) Determined values.

group, the cytoplasmic color of TH immunopositive cells is light brown (Fig. 1f). In addition, the neuromelanin is not observed in the interior and vicinity of TH immunopositive

cells (Fig. 1a-1f). The TH immunopositive cells of each group are counted and analysis, respectively. The results are shown in Figures 3a and 3c and Table II. From the control group to

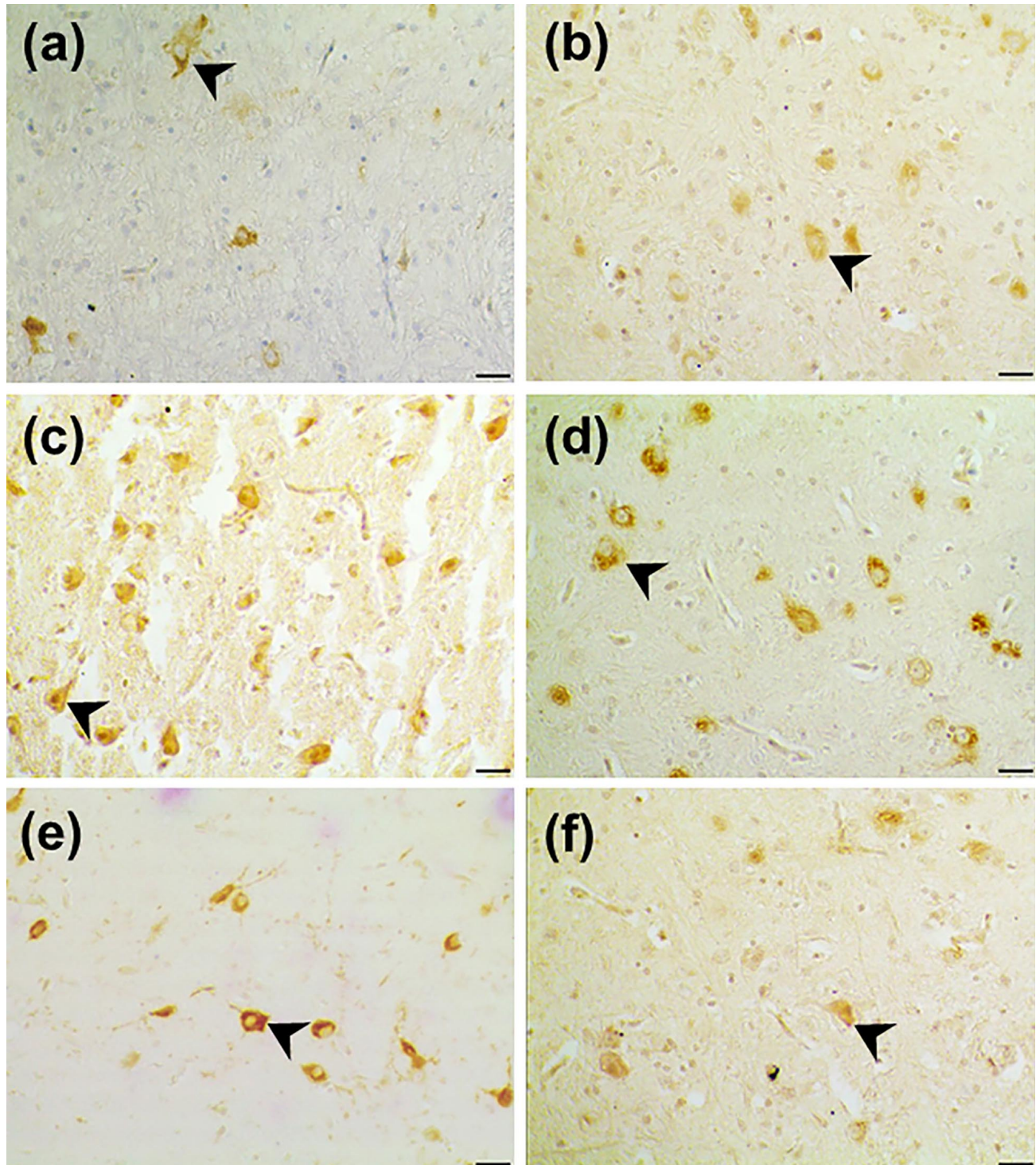


Fig. 1. Microphotographs of tyrosine hydroxylase immunohistochemistry in midbrain of the silky fowl. (a), (b), (c), (d), (e), (f) is control, and 0.2 %, 0.4 %, 0.6 %, 0.8 %, 1.0 % L-tyrosine concentration group, respectively. The tyrosine hydroxylase immunopositive cells are stained brown, but the nuclei are negative, and appear as pale and vesicular. The black arrowheads show representative tyrosine hydroxylase immunopositive cells. Bar= 20 μ m.

the 0.8 % group, the number of TH immunopositive cells is gradually increasing. The number of TH immunopositive cells of the 0.8 % group compared with that of the control group,

the difference is significant ($P < 0.05$). In the 1.0 % group, the number is decreasing. The difference is not significant ($P > 0.05$) compared with the control group.

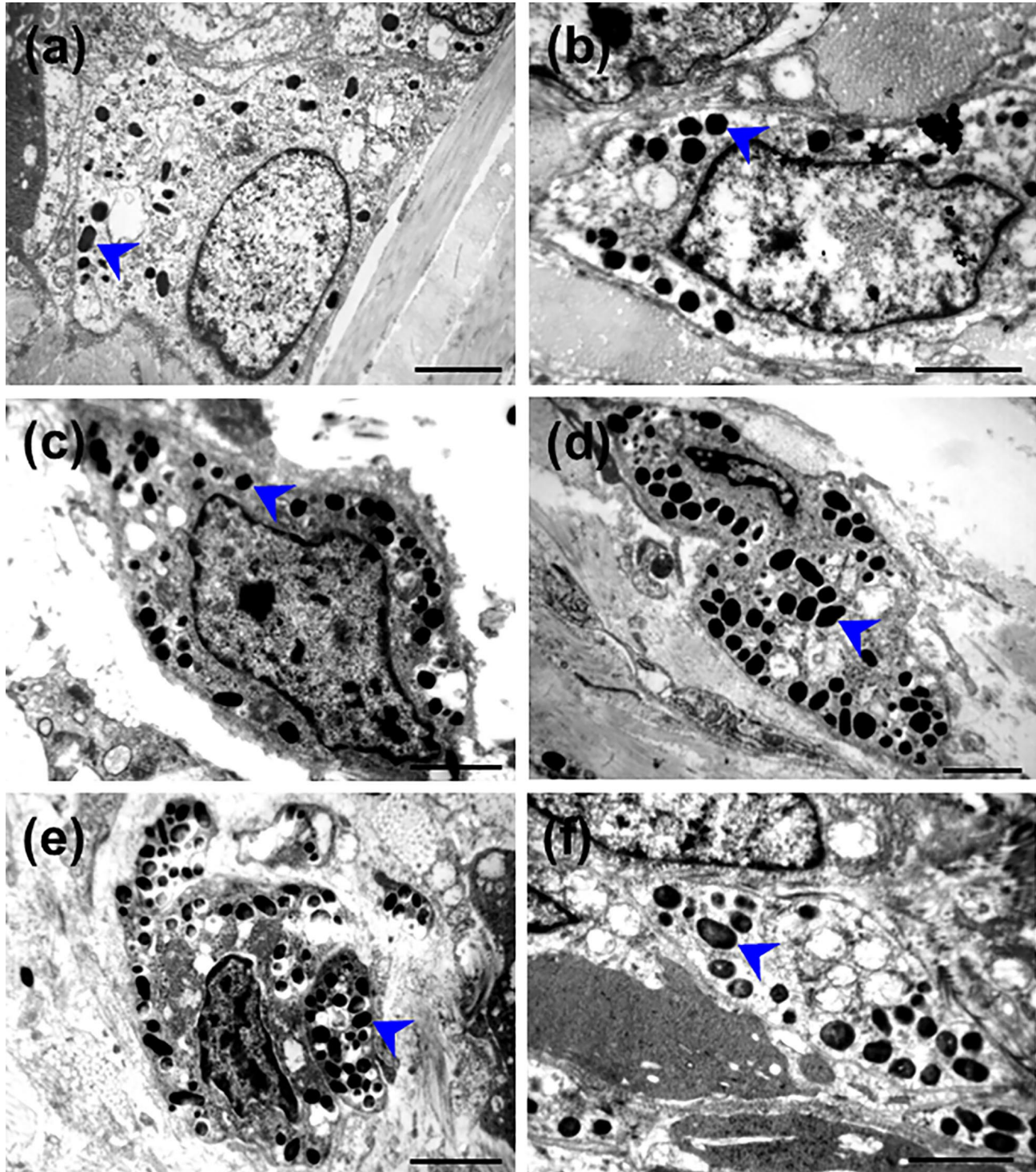


Fig. 2. TEM micrographs of cutaneous melanocytes and their melanosomes. (a), (b), (c), (d), (e), (f) is control, and 0.2 %, 0.4 %, 0.6 %, 0.8 %, 1.0 % L-tyrosine concentration group, respectively. The round and electron-dense melanosomes of melanocytes are observed in each group. The blue arrowheads show representative melanosome in the skin of silky fowl. Bar= 1900 nm.

Table II. The number of the midbrain TH immunopositive cells and cutaneous melanocytic melanosomes in different L-tyrosine concentration groups.

Test group	control	0.2%	0.4%	0.6%	0.8%	1.0%
Number of TH positive cells/visual field	13.1±6.1 ^a	14.7±4.2 ^a	14.9±3.8 ^{ab}	15.4±3.2 ^{ab}	19.1±5.3 ^b	14.1±5.4 ^a
Number of melanosomes/melanocyte	22.4±8.0 ^a	25.0±9.3 ^a	56.2±5.1 ^b	68.8±10.0 ^c	74.0±14.4 ^{dc}	32.2±6.5 ^a

a–d Different letters within the same column indicate significant differences among groups according to Duncan's multiple range ($P < 0.05$).

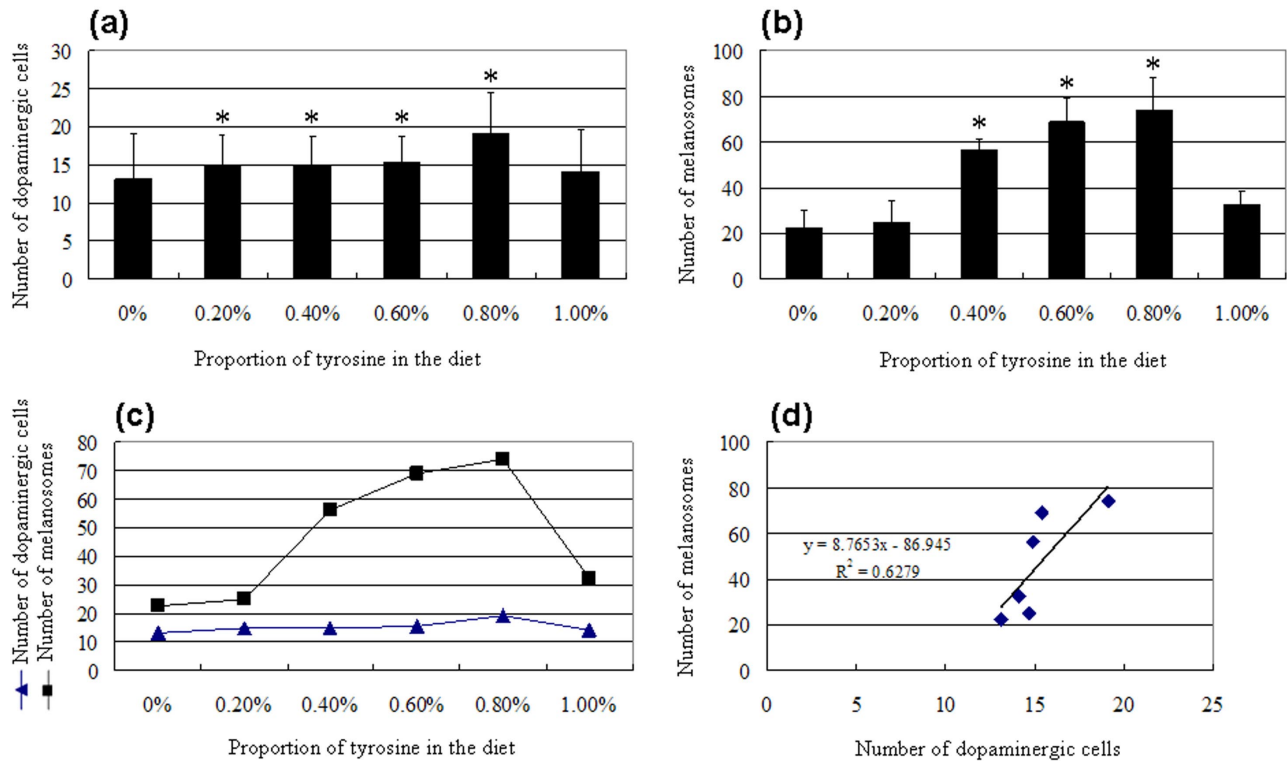


Fig. 3. Effects of different concentrations of L-tyrosine in the diet on the number of midbrain dopamine cells and melanosomes of cutaneous melanocytes. (a) A histogram of L-tyrosine concentrations and the number of dopamine cells. (b) A histogram of L-tyrosine concentrations and the number of melanosomes. (c) Line charts of the number of dopamine cells and melanosomes in different L-tyrosine concentration groups. (d) A correlation of the number of midbrain dopamine cells and that of melanosomes of cutaneous melanocytes. Asterisks (*) indicate the differences are significant ($P < 0.05$) as compared to control.

The effects of L-tyrosine on the number of cutaneous melanocytic melanosomes

By TEM, in the skin, the melanocytes between collagen fibers are observed (Fig. 2a-2f). The cell bodies of melanocytes are irregular. The nuclei of melanocytes exhibit oval, polygonal and flat. Their cytoplasm is electron-lucent. However, the round and oval melanosomes are observed clearly in the cytoplasm, and they are electron-dense. The number of melanosomes per melanocyte is counted and analyzed. The results are shown in Figures 3b and 3c and Table II. From the control group to the 0.8

% group, the number of melanosomes per melanocyte is gradually increasing. In the 0.8 % group, the number of melanosomes per melanocyte reaches the maximum; the mean is 74.0 ± 14.4 . The 0.4 %, 0.6 % and 0.8 % group compared with the control group, the difference is significant ($P < 0.05$), respectively. In the 1.0 % group, the number is decreasing. Compared with the 0.8 % group, the difference is significant ($P < 0.05$), but compared with the control group, the difference is not significant ($P > 0.05$). In addition, the increased number of dopaminergic neurons and that of melanosomes is positively correlated ($R^2 = 0.6279$) (Fig. 3d).

DISCUSSION

In skin, melanin is produced in melanocytes by their unique organelle – melanosomes, which contain the enzyme tyrosinase to initiate melanogenesis from the L-tyrosine (Schallreuter, 2007). L-tyrosine is natural amino acid and is the substrate of melanin biosynthesis. It is catalyzed to L-dihydroxyphenylalanine (L-DOPA), which is converted to dopamine by aminoacid decarboxylase. Furthermore, dopamine is converted to norepinephrine and epinephrine (Daubner *et al.*, 2011). The last three are called catecholamines. L-DOPA and catecholamines serve as primary precursors to the melanin biosynthesis, and regulate positively melanogenesis along with L-tyrosine (Zecca *et al.*, 2001; Gillbro *et al.*, 2004). In addition, in nervous tissues, dopaminergic neurons are mainly located in midbrain. Different from melanocytes containing tyrosinase, dopaminergic neurons express tyrosine hydroxylase (TH). TH is the rate-limiting enzyme for L-DOPA biosynthesis from L-tyrosine, and is a reliable marker for dopaminergic neurons (Arenas *et al.*, 2015). Consequently, TH determines also biosynthesis of catecholamines (Kaushik *et al.*, 2007; Daubner *et al.*, 2011). It has been demonstrated that catecholamines, including dopamine levels, are primarily determined by TH protein levels (Nago-Iwashita *et al.*, 2023). In the present study, TH was expressed in the dopaminergic neurons by IHC investigation. It is suggested that dopaminergic neurons can produce catecholamines by

TH. The neuromelanin is frequently found in the midbrain dopaminergic neurons (Sulzer *et al.*, 2000; Liang *et al.*, 2004). Previous studies have demonstrated that dopaminergic neurons and their TH are involved in neuromelanin biosynthesis by catecholamines (Sulzer *et al.*, 2000; Zecca *et al.*, 2001; Chocarro *et al.*, 2023). Above all, catecholamines can be used as precursors of melanin biosynthesis, regardless of dopaminergic neurons of central nervous system or melanocytes of peripheral skin (Usunoff *et al.*, 2002; d'Ischia *et al.*, 2013).

In the present study, dopaminergic neurons are identified in the midbrain of the silky fowl. Moreover, in the 0-0.8 % L-tyrosine concentration range, the number of dopaminergic neurons increases with the increase of L-tyrosine concentration. L-tyrosine can be transported into specific neurons, for example dopaminergic neurons, by the affinity of amino acid transporters in the brain (Elsworth & Roth, 1997). Previous studies have demonstrated that the raising L-tyrosine concentrations could rapidly stimulate L-tyrosine hydroxylation by TH of dopaminergic neurons, and then stimulates L-DOPA and catecholamine synthesis (Fernstrom, 1990; Tolleson & Claassen, 2012; Nagatsu *et al.*, 2023). These catecholamines could be used as precursors of neuromelanin biosynthesis (Liang *et al.*, 2004). In the present study, however, neuromelanin is not observed in dopaminergic neurons. It is suggested that the increased L-DOPA and catecholamine by dopaminergic neurons could

enter the peripheral blood vessels (Tank & Lee Wong, 2015). Thus, plasma catecholamine is transported into cutaneous melanocytes by blood circulation as precursors to promote peripheral melanin biosynthesis (Gillbro *et al.*, 2004; Schallreuter, 2007).

CONCLUSIONS

Our results demonstrate that, within the proper concentration range, high concentration of L-tyrosine in diet increases the number of dopaminergic neurons in midbrain and melanosome in cutaneous melanocytes in the silky fowl. A highly efficient mechanism for melanin biosynthesis is suggested that the silky fowl could uptake substrate L-tyrosine by dual pathways of midbrain dopaminergic neurons and cutaneous melanocytes (Fig. 4). In addition to L-tyrosine entered

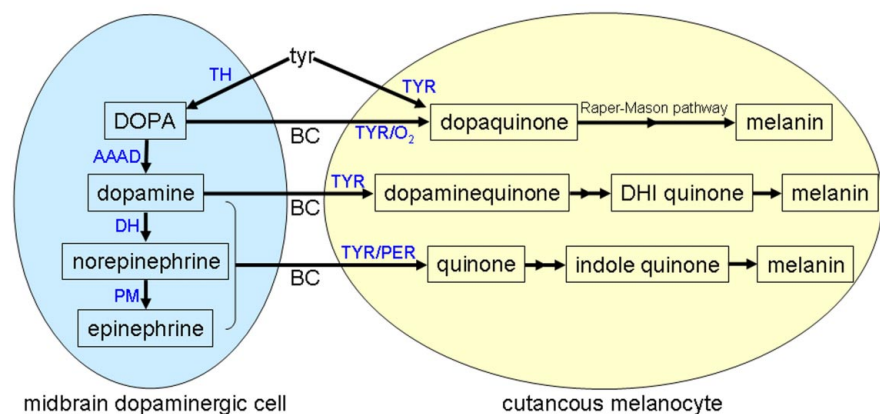


Fig. 4. A schematic diagram of possible metabolic pathways of L-tyrosine (tyr) in the body and its involvement in melanin synthesis. Midbrain dopaminergic neurons uptake tyr, and convert it to L-dihydroxyphenylalanine (L-DOPA) by tyrosine hydroxylase (TH), which is the rate-limiting enzyme of the pathway. L-DOPA is converted to dopamine by aromatic amino acid decarboxylase (AAAD). Dopamine- β -hydroxylase (DH) hydroxylates dopamine to norepinephrine, which is methylated to epinephrine by phenylethanolamine N-methyltransferase (PM). Dopamine, norepinephrine and epinephrine are catecholamines. L-DOPA and catecholamines that produced in the central nervous system are transported into peripheral cutaneous melanocytes by blood circulation (BC) as precursors to participate in melanin biosynthesis. Moreover, cutaneous melanocytes can also uptake directly L-tyrosine to produce melanin. A dual L-tyrosine uptake pathway is present in the silky fowl for efficient biosynthesis of melanin. TYR, tyrosinase; PER, peroxidase; DHI, 5, 6-dihydroxyindole.

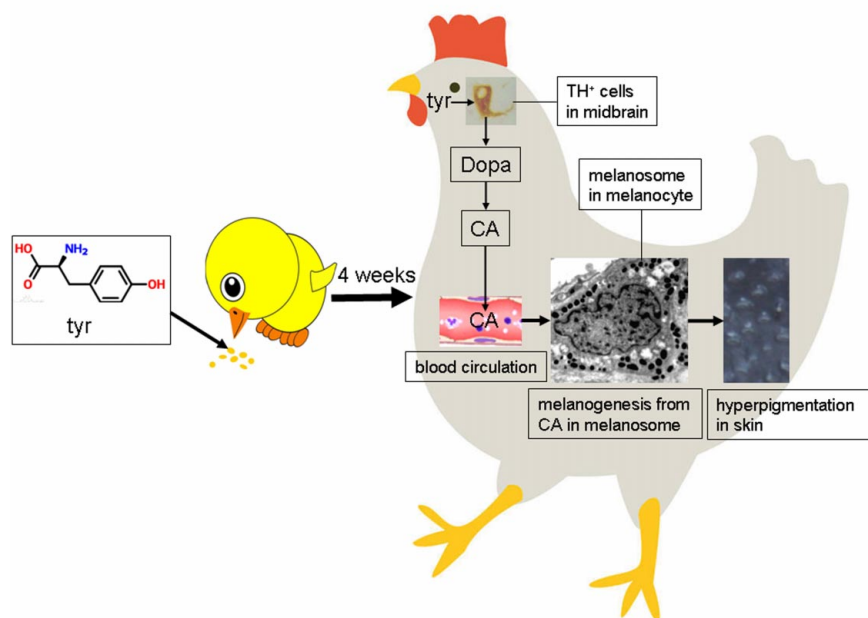


Fig. 5. A schematic diagram of midbrain dopaminergic neurons involved in skin melanogenesis of the chicken. L-tyrosine (tyr) is uptaken by the chickens, and can be catalized to Dopa by Tyrosine Hydroxylase (TH) of midbrain dopaminergic neurons. Moreover, Dopa can be converted to catecholamines (CA). Dopa and CA is transported to melanocytes of skin by blood circulation. Dopa and CA serve as substrates and are biosynthesized melanin by melanosomes of skin. Consequently, midbrain dopaminergic neurons are involved in dietary L-tyrosine promotes cutaneous melanocytic melanosomes.

melanocytes for melanin biosynthesis directly, in the dopaminergic neurons, L-tyrosine is also uptaken and converted to L-DOPA and catecholamines, which are transported into cutaneous melanocytes as precursors for melanin biosynthesis. Consequently, central dopaminergic neurons are involved in peripheral melanogenesis by catecholamine production (Fig. 5). The results provide a new approach and idea to disclose the physiological mechanism of hyperpigmentation in avian.

YE, Y.; CHEN, H.; XIAO, H.; ZHAN, X.; LIU, C.; CHEN, S. & ZHANG, H. Efectos de la L-tirosina dietética sobre las neuronas dopaminérgicas y los melanosomas cutáneos en la gallina sedosa. *Int. J. Morphol.*, 44(4):1141-1148, 2026.

RESUMEN: La gallina sedosa se caracteriza por la hiperpigmentación. Sin embargo, actualmente, el mecanismo fisiológico de la hiperpigmentación no se ha dilucidado por completo. Además, los melanocitos periféricos y las neuronas dopaminérgicas centrales producen melanina y neuromelanina a partir del mismo sustrato —la L-tirosina—, respectivamente, pero la relación entre las neuronas dopaminérgicas y la melanogénesis periférica sigue sin estar clara. En el presente estudio, se administraron diferentes niveles de L-tirosina dietética a distintos grupos de gallinas sedosas. Tras cuatro semanas, se identificaron y cuantificaron las neuronas dopaminérgicas del mesencéfalo y los melanosomas de los melanocitos cutáneos mediante cortes histológicos en parafina, inmunohistoquímica de tirosina

hidroxilasa (TH) y microscopía electrónica de transmisión, respectivamente. En un rango de concentración de 0-0,8 %, la L-tirosina incrementó el número de neuronas dopaminérgicas y melanosomas ($P < 0,05$). Además, se observó una correlación positiva entre el aumento del número de neuronas dopaminérgicas y el de melanosomas ($R^2 = 0,6279$). Los resultados demuestran que la L-tirosina promueve el incremento de las neuronas dopaminérgicas del mesencéfalo y de los melanosomas de los melanocitos cutáneos. La L-tirosina regula positivamente la melanogénesis cutánea a través de las neuronas dopaminérgicas mediante la TH en el pollo sedoso.

PALABRAS CLAVE: Pollo; Célula dopaminérgica; Tirosina hidroxilasa; Melanosoma.

REFERENCES

- Arenas, E.; Denham, M. & Villaescusa, J. C. How to make a midbrain dopaminergic neuron. *Development*, 142(11):1918-36, 2015.
- Bonaventure, J.; Domingues, M. J. & Larue, L. Cellular and molecular mechanisms controlling the migration of melanocytes and melanoma cells. *Pigment Cell Melanoma Res.*, 26(3):316-25, 2013.
- Chocarro, J.; Rico, A. J.; Ariznabarreta, G.; Roda, E.; Honrubia, A.; Collantes, M.; Peñuelas, I.; Vázquez, A.; Rodríguez-Pérez, A. I.; Labandeira-García, J. L.; et al. Neuromelanin accumulation drives endogenous synucleinopathy in non-human primates. *Brain*, 146(12):5000-14, 2023.
- Daubner, S. C.; Le, T. & Wang, S. Tyrosine hydroxylase and regulation of dopamine synthesis. *Arch. Biochem. Biophys.*, 508(1):1-12, 2011.
- d'Ischia, M.; Wakamatsu, K.; Napolitano, A.; Briganti, S.; Garcia-Borron, J. C.; Kovacs, D.; Meredith, P.; Pezzella, A.; Picardo, M.; Sarna, T.; et al. Melanins and melanogenesis: Methods, standards, protocols. *Pigment Cell Melanoma Res.*, 26(5):616-33, 2013.
- Dorshorst, B.; Okimoto, R. & Ashwell, C. Genomic regions associated with dermal hyperpigmentation, polydactyly and other morphological traits in the Silkie chicken. *J. Hered.*, 101(3):339-50, 2010.
- ElObeid, A. S.; Kamal-Eldin, A.; Abdelhalim, M. A. K. & Haseeb, A. M. Pharmacological properties of melanin and its function in health. *Basic Clin. Pharmacol. Toxicol.*, 120(6):515-22, 2017.
- Elsworth, J. D. & Roth, R. H. Dopamine synthesis, uptake, metabolism, and receptors: Relevance to gene therapy of Parkinson's disease. *Exp. Neurol.*, 144(1):4-9, 1997.
- Fernstrom, J. D. Aromatic amino acids and monoamine synthesis in the central nervous system: Influence of the diet. *J. Nutr. Biochem.*, 1(10):508-17, 1990.
- Gillbro, J. M.; Marles, L. K.; Hibberts, N. A. & Schallreuter, K. U. Autocrine catecholamine biosynthesis and the beta-adrenoceptor signal promote pigmentation in human epidermal melanocytes. *J. Invest. Dermatol.*, 123(2):346-53, 2004.

- Hatch, K.; Murphy, E. K.; Cordero, R. J. B. & Iacono, D. Melanin and neuromelanin in humans: Insights across health, aging, diseases, and unexpected aspects of fungal melanogenesis. *Biomolecules*, 16(1):61, 2025.
- Huang, C.; Wei, Y.; Kang, Z.; Zhang, W. & Wu, Y. Transcriptome analysis of skeletal muscles of black-boned chickens, including 2 types (wild and mutated) of Taihe black-boned silky fowl and 1 type (wild) of Yugan black-boned chicken. *Poult. Sci.*, 103(2):103240, 2023.
- Ju, X.; Zhang, M.; Tu, Y.; Ji, G.; Shan, Y.; Liu, Y.; Zhao, W. & Shu, J. Assessment of nutritional value and flavor characteristics of Taihe silky fowl across varieties and marketing ages. *Food Chem. X*, 33:103444, 2025.
- Kaushik, P.; Gorin, F. & Vali, S. Dynamics of tyrosine hydroxylase mediated regulation of dopamine synthesis. *J. Comput. Neurosci.*, 22(2):147-60, 2007.
- Liang, C. L.; Nelson, O.; Yazdani, U.; Pasbakhsh, P. & German, D. C. Inverse relationship between the contents of neuromelanin pigment and the vesicular monoamine transporter-2: Human midbrain dopamine neurons. *J. Comp. Neurol.*, 473(1):97-106, 2004.
- Liao, X.; Shi, X.; Hu, H.; Han, X.; Jiang, K.; Liu, Y. & Xiong, G. Comparative metabolomics analysis reveals the unique nutritional characteristics of breed and feed on muscles in Chinese Taihe black-bone silky fowl. *Metabolites*, 12(10):914, 2022.
- Liu, J.; Gao, H.; Liu, T.; Zhang, T.; Nan, T.; Li, H.; Li, H.; Li, J. & Yuan, Y. The immune regulation of melanin from *Gallus gallus domesticus* Brisson against cyclophosphamide-induced immunosuppression. *Food Sci. Nutr.*, 13(5):e70253, 2025.
- Nagatsu, T.; Nakashima, A.; Watanabe, H.; Ito, S.; Wakamatsu, K.; Zucca, F. A.; Zecca, L.; Youdim, M.; Wulf, M.; Riederer, P. & Dijkstra, J. M. The role of tyrosine hydroxylase as a key player in neuromelanin synthesis and the association of neuromelanin with Parkinson's disease. *J. Neural Transm.*, 130(5):611-25, 2023.
- Nago-Iwashita, Y.; Moriya, Y.; Hara, S.; Ogawa, R.; Aida, R.; Miyajima, K.; Shimura, T.; Muramatsu, S. I.; Ide, S.; Ikeda, K.; *et al.* Overexpression of tyrosine hydroxylase in dopaminergic neurons increased sensitivity to methamphetamine. *Neurochem. Int.*, 164:105491, 2023.
- Qiu, Y.; Ying, J.; Yan, F.; Yu, H.; Zhao, Y.; Li, H.; Xia, S.; Chen, J. & Zhu, J. Novel antiosteoporotic peptides purified from protein hydrolysates of Taihe black-boned silky fowl: By larval zebrafish model and molecular docking. *Food Res. Int.*, 169:112850, 2023.
- Schallreuter, K. U. Advances in melanocyte basic science research. *Dermatol. Clin.*, 25(3):283-91, 2007.
- Sulzer, D.; Bogulavsky, J.; Larsen, K. E.; Behr, G.; Karatekin, E.; Kleinman, M. H.; Turro, N.; Krantz, D.; Edwards, R. H.; Greene, L. A.; *et al.* Neuromelanin biosynthesis is driven by excess cytosolic catecholamines not accumulated by synaptic vesicles. *Proc. Natl. Acad. Sci. U. S. A.*, 97(22):11869-74, 2000.
- Tank, A. W. & Lee Wong, D. Peripheral and central effects of circulating catecholamines. *Compr. Physiol.*, 5(1):1-15, 2015.
- Tolleson, C. & Claassen, D. The function of tyrosine hydroxylase in the normal and Parkinsonian brain. *CNS Neurol. Disord. Drug Targets*, 11(4):381-6, 2012.
- Usunoff, K. G.; Itzev, D. E.; Ovtcharoff, W. A. & Marani, E. Neuromelanin in the human brain: A review and atlas of pigmented cells in the substantia nigra. *Arch. Physiol. Biochem.*, 110(4):257-369, 2002.
- Zecca, L.; Tampellini, D.; Gerlach, M.; Riederer, P.; Fariello, R. G. & Sulzer, D. Substantia nigra neuromelanin: Structure, synthesis, and molecular behaviour. *Mol. Pathol.*, 54(6):414-8, 2001.
- Zhang, Z.; Li, G.; Wei, Y.; Feng, Z.; Fang, L.; Li, M.; Ren, J.; Liu, W. & Gan, J. In vitro immunomodulatory and antioxidant effects of oligopeptides and four characteristic peptides in black-bone silky fowl (*Gallus gallus domesticus* Brisson). *J. Food Biochem.*, 46(12):e14469, 2022.

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