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Nutritional Characteristics and Functions of D-Amino Acids in the Chicken

Shozo Tomonaga[1] and Mitsuhiro Furuse[2]

[1] Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
[2] Laboratory of Regulation in Metabolism and Behavior, Faculty of Agriculture, Kyushu University, Fukuoka 819-0395, Japan

D-Amino acids occur in modest amounts in bacterial proteins and the bacterial cell wall, as well as in peptide antibiotics. Therefore, D-amino acids present in terrestrial vertebrates were believed to be derived from bacteria present in the gastrointestinal tract or fermented food. However, both exogenous and endogenous origins of D-amino acids have been confirmed. Terrestrial vertebrates possess an enzyme for converting certain L-isomers to D-isomers. D-Amino acids have nutritional aspects and functions, some are similar to, and others are different from those of L-isomers. Here, we describe the nutritional characteristics and functions of D-amino acids and also discuss the future perspectives of D-amino acid nutrition in the chicken.

Key words: chickens, D-amino acid, function, L-amino acid, nutrition

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Introduction

One of the main objectives of animal production is the provision of proteins with excellent amino acid composition. Attention has been focused on the 19 L-α-amino acids and glycine necessary for protein synthesis. Therefore, it is essential to obtain sufficient quantities of these amino acids. Although some amino acids are used for protein synthesis, they also have other physiological functions in the free form. The amino acid function can be confirmed regardless of whether it is nutritionally essential or non-essential. For example, L-glutamate (Glu) and L-aspartate (Asp) act as excitatory neurotransmitters while glycine acts as an inhibitory neurotransmitter. L-Leucine (Leu) stimulates protein synthesis (Anthony *et al*., 2000) and suppresses protein degradation (Buse and Reid, 1975) in skeletal muscle. Certain non-coded or non-proteinogenic L-α-amino acids have been used as markers linked to animal production: 3-methyl-L-histidine is an indicator of skeletal protein degradation (Asatoor and Armstrong, 1967) and L-hydroxyproline is an indicator of collagen metabolism (Reddy and Enwemeka, 1996). In addition to these L-α-amino acids, β-amino acids, γ-amino acids, and D-amino acids have many functions in

animals. For example, β-alanine (Tiedje *et al*., 2010) and γ-aminobutyric acid (GABA) act as inhibitory neurotransmitters. In this review, we focused on the role of D-amino acids in chicken physiology and nutrition.

For this review, it is appropriate at first, to discuss certain studies on D-amino acid metabolism. Firstly, D-amino acids are recognized as essential constituents of bacterial peptidoglycans and some antibiotics. D-Amino acids are synthesized enzymatically rather than ribosomal. The primary function of mammalian D-amino acid oxidase (DAAO) and D-Asp oxidase was to detoxify the small amounts of D-amino acids derived from the intestinal bacterial flora (Bender, 2012). Subsequently, serine (Ser), Asp, and alanine (Ala) racemases are identified in the eukaryotes and several marine invertebrates. D-Asp was present in the nervous system of cephalopods and D-Ala in the muscle and hepatopancreas of crustaceans (Bender, 2012). Furthermore, the presence of D-Ser, D-Asp, and D-Ala was confirmed in plasma and brain tissues of both germ-free and specific pathogen-free mice (Kawase *et al*., 2017). These facts suggest that DAAO and D-Asp oxidase are necessary for the D-amino acids produced by intestinal bacteria and those provided by the animals. To understand the role of D-amino acids in nutrition, it is essential to recognize the intestinal amino acid transporters. Transport of most free amino acids from the lumen of the small intestine into the mucosal cells occurs by active processes that transport amino acids against a concentration gradient. These transport mechanisms require energy and show specificity for the L-forms of the amino acids. D-Amino acids are generally absorbed slower than the corresponding L-forms (Scott *et al*., 1982).

The present review aimed to introduce the nutritional

characteristics and functions of each D-amino acid. We focused on and compared their distribution, possible roles, differences between D- and L-forms in chickens and mammals. We also discussed future perspectives of D-amino acid nutrition in chickens.

D-Amino Acids with Side Chains Containing Acidic Groups

D-Asp

D-Asp occurs in the central nervous system of various species, including chickens, pigeons, rats, mice, and humans (Hashimoto *et al*., 1993a, 1995; Kera *et al*., 1996; Morikawa *et al*., 2001; Neidle and Dunlop, 1990). Although D-Asp is present in selected neuronal populations in the brain, it is concentrated mainly in glands, especially the epinephrine-containing cells of the adrenal medulla, the posterior pituitary, and the pineal gland (Dunlop *et al*., 1986; Hashimoto *et al*., 1993b; Schell *et al*., 1997). Neidle and Dunlop (1990) measured free D-Asp in White Leghorn chicken embryos. In each tissue examined, the maximum D-Asp level was found at a specific time during development. In the embryo brain, the highest D-Asp was 9% of total L- and D-Asp at 11 days of incubation and 20% at 13 days of incubation in the retina. In addition, the total D-Asp content of the egg, including the embryo, increased from 45 nmol/egg on day-1 to 159 nmol/egg on day-12. Hence, suggesting the release of D-Asp from the bound form or *de novo* synthesis is a continual process during development.

Zachar *et al*. (2018) determined the extracellular concentration of D-Asp in microdialysis samples from the brain regions (medial striatum, intermediate medial mesopallium, arcopallium, and cerebellum) of freely moving Hunnia broiler chicks. D-Asp decreased during the first week of age, with no considerable regional- or learning-related variation. In layer chicks, Erwan *et al*. (2012) carried out behavioral experiments under socially isolated stressful conditions exacerbated by the use of corticotrophin-releasing hormone (CRH). Intracerebroventricular (i.c.v.) injection of both L- and D-Asp induced sedative effects under an acutely stressful condition. L-Asp (but not D-Asp) increased the time spent in a sleeping posture. These results indicate that both L- and D-Asp, when present in the brain, could induce a sedative effect, although the mechanism for hypnosis in neonatal chicks may be different for L-Asp compared to D-Asp. Later, Erwan *et al*. (2014b) investigated the involvement of the N-methyl-D-aspartate (NMDA)-type of L-Glu receptor to identify the receptor-mediated function of L- and D-Asp in layer chicks. As shown in Fig. 1, the L-Asp-mediated sedative and hypnotic effects were blocked by co-administration of (+)-MK-801, an antagonist of NMDA receptors. Importantly, the sedative effects induced by D-Asp shifted to hypnotic effects with co-administration of (+)-MK-801. Taken together, L-Asp could induce sedative and hypnotic effects for stress behaviors through the NMDA receptor. However, the attenuation of stress behaviors by D-Asp could be due to the simultaneous involvement of receptors other than the NMDA receptor. D'Aniello *et al*. (2011) suggested
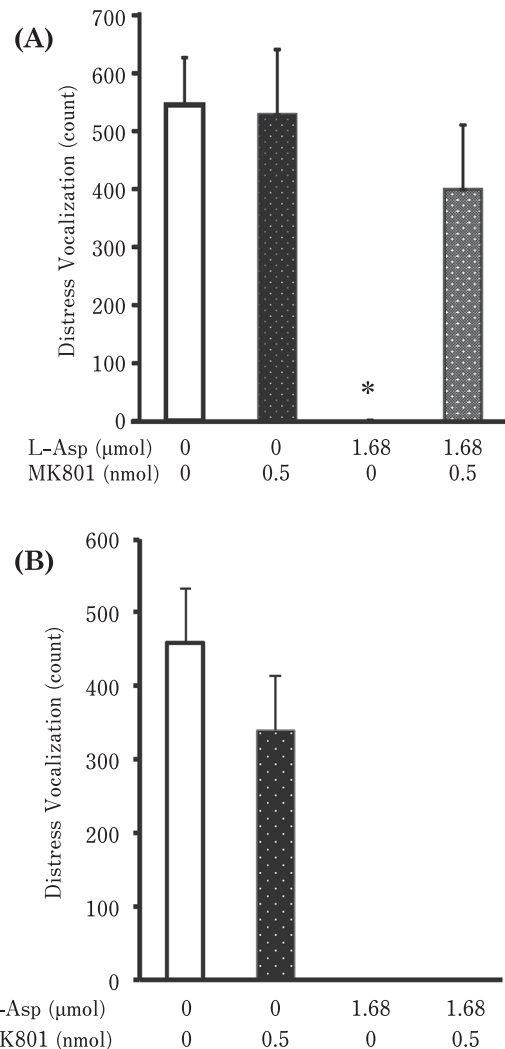

Fig. 1. **(A) Effects of i.c.v. injection of saline, (+)-MK-801 maleate (0.50 nmol), L-Asp (1.68 μmol) or L-Asp (1.68 μmol) plus (+)-MK-801 maleate (0.50 nmol). (B) Effects of i.c.v. injection of saline, (+)-MK-801 maleate (0.50 nmol), D-Asp (1.68 μmol) or D-Asp (1.68 μmol) plus (+)-MK-801 maleate (0.50 nmol) on the total number of distress vocalizations during 10 min isolation in 5–6-day-old layer chicks.** Results presented as mean$\pm$S.E.M. *Significantly different from other treatments at $P<0.05$. Reproduced from Erwan E, Chowdhury VS, Nagasawa M, Goda R, Otsuka T, Yasuo S, Furuse M. Central injection of L- and D-aspartate attenuates isolation-induced stress behavior in chicks possibly through different mechanisms. European Journal of Pharmacology, 736: 138–142. 2014b, with permission from Elsevier.

that rat postsynaptic membranes possess a specific type of receptor for D-Asp, different from that which triggers L-Glu. In contrast, D-Asp could also be the precursor for the endogenous synthesis of NMDA (D'Aniello *et al*., 2000). The synthesis of NMDA may be modified by i.c.v. injection of D-Asp.

Concerning the nutritional aspects of D-Asp, Maruyama *et al*. (1972) reported that in chicks produced from mating male New Hampshire with female Single Comb White Leghorn, the presence of 2% of D-Asp in the diet significantly depressed growth, whereas 6% L-Asp did not. When L-Asp replaced D-Asp, after one week, the growth rate and feed consumption increased rapidly and approached the values of the control birds. Erwan *et al*. (2013) confirmed that oral administration of several doses of L-Asp increased the concentration of L-Asp linearly, but not of D-Asp, in the plasma of layer chicks. Oral L-Asp administration resulted in a slight increase of D-Asp levels in the telencephalon, but not in the diencephalon. However, food intake did not change significantly with L-Asp doses. On the other hand, D-Asp strongly and dose-dependently inhibited food intake during the 2-h administration period. Oral D-Asp increased D-Asp levels in the plasma and diencephalon, but resulted in no significant changes in L-Asp. In addition to food intake and growth, body temperature was also affected by D-Asp. According to Erwan *et al*. (2014a), oral administration of D-Asp (but not of L-Asp) linearly decreased the rectal temperature in layer chicks. Importantly, orally administered D-Asp led to significant reduction in body temperature in chicks, even under high ambient temperatures. However, centrally administered D-Asp did not have considerable influence on the body temperature in layer chicks. As for the plasma metabolites and catecholamines, orally administered D-Asp decreased triacylglycerol and uric acid concentrations and increased glucose and chlorine concentrations, but did not alter plasma catecholamines. These results suggest that oral administration of D-Asp, different from L-Asp, may play a significant role in reducing body temperature under both normal and high ambient temperature conditions. The changes in plasma metabolites further indicate that D-Asp may contribute to the regulation of metabolic activity in layer chicks. However, these results suggested nutritional differences between L-Asp and D-Asp.

D-Glu

D-Glu and -Asp are acidic and vital amino acids. Compared to D-Asp, the information for D-Glu is limited. Maruyama *et al*. (1975) reported that chicks derived from mating male New Hampshire with female Single Comb White Leghorn, tolerated nearly 15% of dietary L-Glu with no growth retardation. However, 3.75% of D-Glu levels resulted in growth suppression at the end of the 2-week experiment. Free ammonia increased in both the liver and kidney when D-Glu was included in the diet.

D-Amino Acids with Side Chains Containing Hydroxylic Groups

D-Ser

D-Ser is physiologically relevant as a co-agonist of NMDA receptors in mammals (Wolosker *et al*., 2008). To our knowledge, however, it is unclear whether D-Ser has a similar physiological function in chickens. Nagata *et al*. (1994) confirmed that cerebrum in White Leghorn chickens (adult, 2.5−3 kg) contains much lower amount of D-Ser

compared to Wistar rats (male, 9 weeks old, 240−260 g), and BALB/cA mice (female, 8 weeks old) (means (nmol/g wet tissue)±SEM: chickens, 7.0±0.7; mice, 520±98; rats, 395 ±74, respectively). It is worth investigating whether the differences in levels are associated with different physiological roles in animals.

Chicks are comfortable in crowded conditions, but become stressed when isolated. This social separation stress increases spontaneous activity in chicks as well as their distress vocalizations (Saito *et al*., 2005). According to Asechi *et al*. (2006), in layer chicks, i.c.v. injection of L-Ser significantly decreased the number of distress vocalizations, but D-Ser did not. In the L-Ser treated group, the time for active wakefulness decreased significantly and increased substantially for sleeping posture compared to the control group. However, in the D-Ser treated group, no behavioral differences were observed compared to the control group, and sleeping posture was absent. I.c.v. injection of several glutamate receptor agonists was administered to chicks under social separation stress. In layer chicks, L-Glu dose-dependently induced a hypnotic effect (Yamane *et al*., 2009b). Although NMDA induced a sedative effect, the potency of NMDA for sleep-like behavior was lower than L-Glu (Yamane *et al*., 2009b). Kleckner and Dingledine (1988) suggested that, for activation of the NMDA receptor-channel complex, two different agonists are required. D-Ser alone or NMDA alone showed negligible or minor effects when administered centrally (Asechi *et al*., 2006; Yamane *et al*., 2009b). These results suggest that in contrast to L-Ser, D-Ser alone did not induce anti-stress effects under social isolation stress. Further studies focusing on the relationship between NMDA receptors and D-Ser are needed.

Dietary effects of D-Ser have not been investigated thoroughly. Sugahara *et al*. (1967) studied the effect of replacement of L-Ser with D-Ser (1.9%) in purified diets containing crystalline amino acids as the sole source of nitrogen, on White Leghorn chick growth. They confirmed that D-Ser had no adverse effects on growth. On the other hand, D-Ser in drinking water (350 mg/kg, for five weeks) induced antidepressant activity in mice (Otte *et al*., 2013). In addition, D-Ser in drinking water caused dysfunction of insulin secretion (Suwandhi *et al*., 2018). Therefore, the relationship between dietary D-Ser and various health conditions in chickens should be investigated in the future.

D-Amino Acids with Aliphatic Side Chains

D-Ala

In chicks produced from the mating of male New Hampshire with female Single Comb White Leghorn, 1.5% of dietary D-Ala caused significant growth depression, but not 2% L-Ala or 2% D-Ser. When the amino acid diet contained 4.4% of other D-amino acids, 1.25% of D-Ala also caused growth depression (Maruyama *et al*., 1972).

D- Leu

Among the branched-chain amino acids (BCAAs), L-Leu markedly stimulates protein synthesis (Anthony *et al*., 2000) and suppresses protein degradation (Buse and Reid, 1975) in

skeletal muscles. The mechanism by which L-Leu stimulates protein synthesis in the skeletal muscle is unclear. L-Leu stimulated the phosphatidyl-inositol 3-kinase (PI3K) (Peyrollier *et al.*, 2000; Tesseraud *et al.*, 2003) and the mTOR kinase (Anthony *et al.*, 2000). In contrary, proteolysis suppression by L-Leu does not involve the mTOR signaling pathway (Mordier *et al.*, 2000; Kanazawa *et al.*, 2004; Nakashima *et al.*, 2005). L-Leu metabolite α-ketoisocaproate (α-KIC), suppresses proteolysis in incubated skeletal muscles, suggesting that transamination of L-Leu plays a vital role in the inhibitory effect of proteolysis (Tischler *et al.*, 1982). Nakashima *et al.* (2007) compared the effects of L-Leu, D-Leu, and α-KIC on myofibrillar proteolysis in the skeletal muscle of layer chicks. As a result, D-Leu and α-KIC suppress proteolytic-related genes, resulting in a decrease in myofibrillar proteolysis while L-Leu is notably less effective in the skeletal muscle of the chicks, which may be explained by the conversion of D-Leu to α-KIC.

Sugahara *et al.* (1967) studied the effect of replacing L-Leu with D-Leu (2.3%) in purified diets containing crystalline amino acids as the sole nitrogen source on White Leghorn chicks' growth. They confirmed that D-Leu replacement caused distinct growth retardation; it had some nutritional value, even though it was less than L-Leu (Sugahara *et al.*, 1967).

D-Amino Acids with Side Chains Containing Basic Groups and Their Metabolites

D-Arginine (Arg)

Nitric oxide (NO) is one of the gaseous reactive oxygen species (ROS) that functions as a signaling factor in many physiological and pathophysiological conditions in animals. L-Arg is a substrate for NO synthase, and D-Arg is used as the inactive substrate of NO synthase in mammals; some studies have used D-Arg in chickens for this purpose. Adams *et al.* (1994) used D-Arg to investigate whether endogenous NO production is linked to 1,25-dihydroxy vitamin D$_3$ synthesis in the chick myelomonocytic cell line HD-11. Gwee *et al.* (1995) applied D-Arg to study the involvement of L-Arg-NO pathway in the nerve-evoked relaxant responses of the serotonin precontracted chick isolated upper esophagus. Ungureanu-Longrois *et al.* (1997) used D-Arg to investigate the effect of endogenous NO on contractile function of cultured chick embryo ventricular myocytes.

Sugahara *et al.* (1967) studied the effect of replacement of L-Arg-HCl with D-Arg-HCl (1.9%) in purified diets containing crystalline amino acids as the sole nitrogen source on the growth of White Leghorn chicks. They concluded that the nutritional value of D-Arg-HCl was negligible, because no difference in growth between L-Arg-HCl lacking group and D-Arg-HCl added group was observed.

Published information on D-Arg in physiology and nutrition of the chicken is minimal. Nutritional functions of D-Arg, focusing on NO synthesis will be addressed in the future.

D-Lysine (Lys)

According to Grove and Roghair (1971), the Leghorn-type chicken actively metabolized D-Lys and L-pipecolic acid (PA) in contrast to the rat. The data show that D-Lys is degraded via conversion to L-PA, α-aminoadipate, α-keto-adipate, and eventually to CO_2. The α-nitrogen atom of D-Lys is the first one removed from the carbon skeleton by this route. Broiler chicks (Newman and Sands, 1983), given a choice of diets prepared with an adequate quantity of L-Lys or the same dose of D-Lys, preferred the L-Lys diet, but did not ingest sufficient amount of L-Lys to reach standard growth. These observations indicate that chicks can discern the presence of L-Lys in diets or separately, but cannot ingest adequate quantity to maximize growth potential. A diet prepared with D-Lys was more acceptable than one devoid of Lys, suggesting that chickens may have some sensory recognition for Lys.

D-PA

L-PA is a primary metabolic intermediate of L-Lys in the White Leghorn chick brain (Nomura *et al.*, 1978). The major pathway of L-Lys metabolism includes the intermediate saccharopine, but this pathway predominates in the liver and is not very active in the human brain (Hutzler and Dancis, 1968). Therefore, the L-PA pathway may be particularly important in the brain.

The PA exists as L- and D-enantiomers. L- and D-PA has also been found in human physiological fluids such as plasma, urine, and cerebrospinal fluid (Armstrong *et al.*, 1993a, b). Takagi *et al.* (2001) administered L-PA i.c.v. to examine its effect on food intake in broiler chicks (Cobb); while the i.c.v. injection of L-PA suppressed food intake, a higher concentration than D-PA was required during a short period (Fig. 2). L- and D-PA may have different metabolic mechanisms (Dancis and Hutzler, 1981). Takagi *et al.* (2001) observed an increase in sleep-like response following the i.c.v. injection of L-PA. Takagi *et al.* (2003) investigated the association of induction of sleep-like behaviors by L-PA with GABA neurotransmission and the GABA subtype receptor activation mediates this effect in broiler and layer chicks. L-PA increased the duration of sleeping posture time and decreased the waking posture time, and the GABA-A antagonist picrotoxin attenuated the effects. Similarly, the GABA-B antagonist CGP54626 also attenuated the effects of L-PA. Together, these findings suggest that in neonatal chicks, the induction of sleep-like response by L-PA involved the GABAergic system. However, the details of the function of D-PA remain unknown.

D-Ornithine (Orn)

The effects of i.c.v. injection of L-Orn, L-citrulline, and D-Orn were compared in layer chicks under isolation-induced stress (Kurauchi *et al.*, 2009). L-Orn greatly attenuated the stress response and induced sedative and hypnotic effects. However, D-Orn weakly attenuated the stress responses, while L-citrulline did not. Involvement of L-Orn, its other metabolites, or both were investigated for sedative and hypnotic effects under social separation stress (Kurauchi *et al.*, 2010). The effects of i.c.v. injection of L-Orn and polyamines (putrescine, spermidine, and spermine) were compared in chicks. L-Orn significantly attenuated the stress
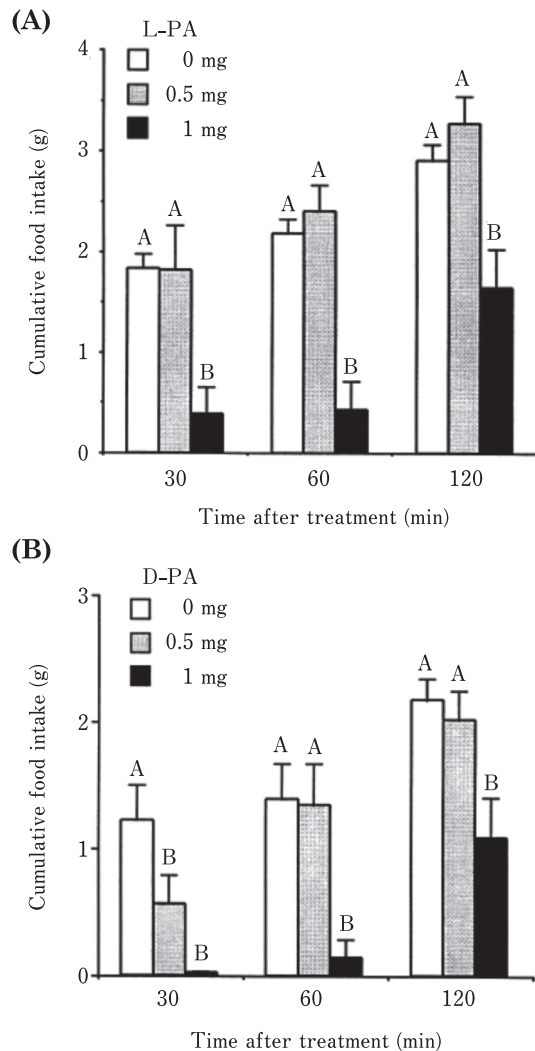
(A)

(B)

Fig. 2. **Cumulative food intake in chicks with i.c.v. administered saline, (A) L-PA (0.5 and 1 mg) or (B) D-PA (0.5 and 1 mg) after 3 h fasting.** Means with a different letter at each time are significantly different at $P<0.05$. Values are means$\pm$S.E.M. Reproduced from Takagi T, Ando R, Ohgushi A, Yamashita T, Dobashi E, Hussain-Yusuf H, Onodera R, Bungo T, Sato H, Furuse M. Intracerebroventricular injection of pipecolic acid inhibits food intake and induces sleeping-like behaviors in the neonatal chick. Neuroscience Letters, 310: 97–100. 2001, with permission from Elsevier.

response and caused sedative and hypnotic effects. Among the polyamines, only putrescine attenuated distress vocalizations but did not induce sleep. It was concluded that the sedative and hypnotic effect of L-Orn was induced primarily by L-Orn. Later, Kurata et al. (2011) showed L-Orn function by the activation of GABA-A receptor. These results indicate differential effects of L-Orn and D-Orn in the brain of chickens. However, from the nutritional point of view, dietary L-Orn or D-Orn has not been well investigated in

chickens.

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D-Amino Acids with Side Chains Containing Sulfur Atoms

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D-Cysteine (Cys)

Excitatory amino acid receptor potency and subclass specificity of sulfur-containing amino acids were investigated (Pullan et al., 1987) in two excitatory amino acid receptor functional assays and receptor binding assays. The sulfur-containing amino acids, L- and D-cysteate, L-Cys, L- and D-Cys sulfinate, L- and D-Cys-S-sulfate, L-cystine, L- and D-homocysteate, L- and D-homocysteine sulfinate, L-homocysteine, L-serine-O-sulfate, and taurine were tested. The majority of the sulfur-containing amino acids tested showed the highest affinities for the NMDA and L-2-amino-4-phosphonobutanoate binding sites. Most of the sulfur-containing amino acids inhibited sodium-dependent L-[^{3}H] glutamate binding. The L isomers were more potent inhibitors than the D isomers, with substantial difference seen for L- and D-Cys-S-sulfate and negligible selectivity displayed for L- and D-Cys sulfinate.

Yamane et al. (2009a) reported that the i.c.v. injection of L-Cys and D-Cys decreased both distress vocalization and spontaneous activity induced by isolation in layer chicks. However, the two Cys isomers induced different behaviors. L-Cys increased sleep-like behavior while D-Cys caused abnormal behavior, including syncope as well as sleep-like behavior. In conclusion, while both L-Cys and D-Cys caused a sedative effect when injected i.c.v., D-Cys caused abnormal behavior and may be detrimental to neonatal chicks. According to the results mentioned above (Pullan et al., 1987), abnormal response caused by central D-Cys might not be through the Glu sub-receptors. Both L-Cys and D-Cys can stimulate hydrogen sulfide (H$_2$S) via different mechanisms in mammals (Shibuya and Kimura, 2013). H$_2$S functions in the regulation of neuronal activity, vascular tension, release of insulin, and protection of certain tissues from ischemic insults (Shibuya and Kimura, 2013). Therefore, efforts focusing on H$_2$S may be useful to discern the differences in physiological and nutritional effects of L-Cys and D-Cys in chickens.

D-Methionine (Met)

In chickens, L-Met is absorbed by an efficient active transport mechanism. In contrast, D-Met absorption by the same transporter is less efficient. D-Met oxidase, which converts D-Met to the keto analog, is quite active (Scott et al., 1982). Kuzmicky et al. (1977) compared three Met sulfoxides and DL-Met in White Rock broiler chickens. The availability of D-, DL-, and L-Met sulfoxide were 52, 59, and 77%, respectively. The differences in the availability of the three sulfoxides and DL-Met were significant. Both DL- and D-Met sulfoxide were available less significantly than L-Met sulfoxide. Baker (1979) evaluated the efficacy and toxicity of the L- and D-isomers of N-acetylmethionine when fed as a source of methionine in crystalline amino acid diets, using growth assays in chicks from crossing male New Hampshire with female Columbian. N-Acetyl-L-Met had a

Met-sparing value of 100% and N-acetyl-D-Met a value of zero. Isosulfurous levels of excess L-Met or N-acetyl-L-Met were equally growth depressing, but L-Met elevated spleen iron deposition to a greater extent than N-acetyl-L-Met. N-acetyl-D-Met in excess showed slight growth depression, entirely due to decrease in voluntary food intake. Baker and Boebel (1980) evaluated the relative efficacy of the D- and L-isomers of Met and 2-hydroxy-4-(methylthio-butyrate) Ca (OH-Met) in chicks from crossing male New Hampshire with female Columbian. With crystalline amino acid diets, D-OH-Met was decisively superior to L-OH-Met as a source of Met activity. The D-isomer of OH-Met was also notably more toxic than the L-isomer, as measured by the magnitude of growth depression and severity of hemolytic anemia. DL-OH-Met was intermediate in toxicity between the pure D- and L-isomers. These results suggest that chickens can utilize D-Met similarly to L-Met, but the efficacy is lower.

D-Amino Acids with Side Chains Containing Aromatic Rings

D-Tryptophan (Trp)

Morrison *et al.* (1956) showed utilization of the D-Trp without the microflora assistance in chicks produced from mating the male New Hampshire with female Columbian and suggested that inefficient absorption results in a very high requirement for orally administered D-Trp. The nutritive values of DL- and D-Trp relative to the L-Trp were 47 and 21%, respectively in White Leghorn chicks (Ohara *et al.*, 1980a). Conversion of D-Trp to the L-Trp was seen in the rat but was absent in chicks. Under similar conditions, D-Trp was measured in the urine of rats and chicks. In rats, of the amount administered only 1% of D-Trp was excreted at most, while most of the D-Trp was excreted (Ohara *et al.*, 1980b) in chicks. In White Leghorn chicks, the relative biological utilization of DL- and D-Trp compared to L-Trp was approximately 55 and 15%, respectively (Ohara and Ariyoshi, 1979). L- and DL-tryptophan were compared to their ability to replace niacin in the diet of the chick. L-Trp was found to be approximately twice as active as DL-Trp when the diet contained glucose as the source of carbohydrate. When starch replaced glucose in the diet, the D-Trp was of some value. The different D-Trp values in starch vs. glucose were due to changes in the bacterial flora of the digestive tract since the D-Trp value decreased by the addition of sulfasuxidine to the diet.

Zamenhof and Ahmad (1979) investigated the effect of early addition of exogenous nutrients on brain growth in White Leghorn chick embryo. The nutrients were introduced into the chorioallantoic membrane on day six or seven of embryonal life. The cerebral hemispheres examined at the end of neuronal proliferation for the following parameters: weight, DNA content (index of cell number), and protein content. D-Trp (slower transport) was inactive, but L-Trp produced significant inhibition. L-Trp inhibited probably by creating amino acid imbalance and interfering with the transport of other amino acids.

L- and D-Trp were compared under CRH-augmented social isolation stress (Yoshida *et al.*, 2015) in layer chicks. When comparing the efficacy between L- and D-Trp against stress behavior, both exhibited a similar effect and quickly suppressed distress vocalizations (Fig. 3). However, the difference in mechanisms of action between the two Trp is not understood.

From these results, even though the nutritional value of D-Trp is well characterized, it is unclear whether it is physiologically relevant.

D-Amino Acids with Side Chains Containing Imino Group

D-Proline (Pro)

Cherkin and Van Harreveld (1978) showed the importance of the L-configuration in White Leghorn chickens for the effects of L-Pro on memory and spreading depression, but not D-Pro and L-azetidine-2-carboxylic acid (L-A2C, the lower homolog of L-Pro). L-Pro, D-Pro, six Pro analogs including two homologs (L-A2C and DL-PA), and five other compounds were examined for their effects on spreading depression and their amnestic and electrophysiological effects (Van Harreveld *et al.*, 1980). L-Pro, L-baikiain, DL-3, 4-dehydroproline, and L-4-hydroxyproline all reduced the incidence of spreading depression in the chick retina and proved to be amnestic. D-Pro, L-pyroglutamic acid, L-A2C, DL-PA, L-Glu diethyl ester, L-isoleucine, and L-norleucine, neither depressed spreading depression nor caused retrograde amnesia. L-Prolyl-L-Pro and L-glutamine did not reduce spreading depression at low concentrations but had significant amnestic effects. None of the listed compounds induced EEG disturbances. Implications for memory mechanisms are discussed in the light of these results.

The action of Pro and its Glu antagonist analog was investigated in the brain tissue of the White Leghorn chicks (Pico *et al.*, 1983). Brain slices were incubated in low concentrations of L-Pro, D-Pro, DL-3,4-dehydro-Pro, L-prolyl-L-Pro, or avian physiological salt solution before induction of depolarization by application of 45 mM K^+. Glu was determined in the efflux material collected both before and after tissue stimulation. The release of endogenous Glu was inhibited significantly by exposure to L-Pro, DL-3, 4-dehydroproline, and L-prolyl-L-Pro, but not to D-Pro.

Two- and five-day-old White Leghorn chicks were injected i.c.v. with D-Pro and L-Pro at 345 μg or 690 μg. D-Pro produced convulsions and lethality, but was non-amnestic, whereas the naturally occurring L-Pro isomer, was non-convulsant and non-toxic but amnestic. D-Pro produced convulsions were accompanied by reduced high frequency in the electroencephalogram and increased slow-wave activity (Cherkin *et al.*, 1978). Hamasu *et al.* (2010) compared the central effects of L-Pro, D-Pro, and trans-4-hydroxy-L-Pro with a lower level (0.5 μmol (57.5 μg)/10 μl) by using the acute stressful model with neonatal layer chicks. All compounds induced the sedative and hypnotic effects. However, plasma corticosterone release under isolation stress was attenuated only by L-Pro. To understand the mechanism
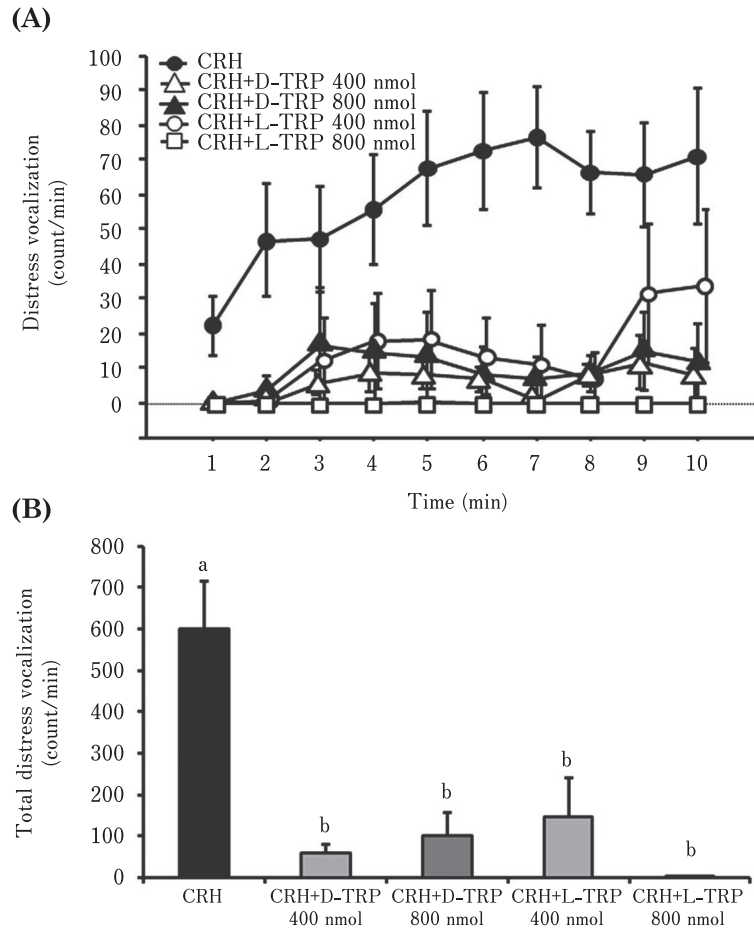
(A)

(B)

Fig. 3. **Effect of i.c.v. injection of CRH or CRH co-injected with L- or D-Trp on time course (A), and total distress (B) vocalizations during 10 min isolation in chicks.** Results are expressed as mean±S.E.M. CRH＝ corticotropin-releasing hormone. Reproduced from Yoshida J, Erwan E, Chowdhury VS, Ogino Y, Shigemura A, Denbow DM, Furuse M. Comparison of centrally injected tryptophan-related substances inducing sedation in acute isolation stress-induced neonatal chicks. Pharmacology, Biochemistry, and Behavior, 129: 1−6. 2015, with permission from Elsevier.

by which L-Pro and D-Pro induce sedative and hypnotic effects, the contribution of the strychnine-sensitive glycine receptor (glycine receptor) and NMDA receptor was investigated further. The glycine receptor antagonist strychnine was co-injected i.c.v. with L-Pro or D-Pro. The suppression of isolation-induced stress behavior by D-Pro was attenuated by strychnine. However, the suppression of stress behavior by L-Pro was not attenuated. The NMDA receptor antagonist (+)-MK-801 was co-injected i.c.v. with L-Pro. The suppression of stress behavior by L-Pro was attenuated by (+)-MK-801. The above data show differential induction of sedative and hypnotic effects through NMDA and glycine receptors by L-Pro and D-Pro, respectively.

Collectively these results indicate the effect of D-Pro is different from L-Pro. However, its physiological importance is unclear.

Conclusions and Future Perspectives

We acquired substantial information on D-amino acids in chickens from the published literature. However, research on D-amino acids in chickens is limited. To understand the physiological and nutritional importance of D-amino acids, both endogenously synthesized and exogenously available D-amino acids should be investigated more precisely. Because of the availability of novel sensitive and accurate analytical methods (Ishii *et al*., 2018; Nakano *et al*., 2019), their application to poultry science will be beneficial. Not only tissues and body fluids, but also cecal content should be investigated to identify the involvement of gut microbiota. To date, it is unknown whether gut microbiota in chickens produces D-amino acids, and if it is physiologically active or not. Hence, studies using germ-free chickens, with proven

utility in the nutritional science of chickens (Furuse and Okumura, 1994), are necessary. In addition, identification of mechanisms underlying the pharmacological effects of D-amino acids may be useful for poultry science. For example, the relationship between D-Cys and H_2S should be investigated, because H_2S generation from D-Cys is expected to be beneficial for mammals (Shibuya and Kimura, 2013). In conclusion, further studies are required to understand the nutritional characteristics and functions of D-amino acids in the chicken.

Acknowledgments

References

Adams JS, Ren SY, Arbelle JE, Clemens TL and Shany S. A role for nitric oxide in the regulated expression of the 25-hydroxy-vitamin D-1-hydroxylation reaction in the chick myelomonocytic cell line HD-11. Endocrinology, 134: 499‒502. 1994.

Anthony JC, Yoshizawa F, Anthony TG, Vary TC, Jefferson LS and Kimball SR. Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. Journal of Nutrition, 130: 2413‒2419. 2000.

Armstrong DW, Gasper M, Lee SH, Zukowski J and Ercal N. D-amino acid levels in human physiological fluids. Chirality, 5: 375‒378. 1993a.

Armstrong DW, Zukowski J, Ercal N and Gasper M. Stereochemistry of pipecolic acid found in the urine and plasma of subjects with peroxisomal deficiencies. Journal of Pharmaceutical and Biomedical Analysis, 11: 881‒886. 1993b.

Asatoor AM and Armstrong MD. 3-Methylhistidine, a component of actin. Biochemical and Biophysical Research Communications, 26: 168‒174. 1967.

Asechi M, Tomonaga S, Tachibana T, Han L, Hayamizu K, Denbow DM and Furuse M. Intracerebroventricular injection of L-serine analogs and derivatives induces sedative and hypnotic effects under an acute stressful condition in neonatal chicks. Behavioural Brain Research, 170: 71‒77. 2006.

Baker DH. Efficacy of the D- and L-isomers of N-acetylmethionine for chicks fed diets containing either crystalline amino acids or intact protein. Journal of Nutrition, 109: 970‒974. 1979.

Baker DH and Boebel KP. Utilization of the D- and L-isomers of methionine and methionine hydroxy analogue as determined by chick bioassay. Journal of Nutrition, 110: 959‒964. 1980.

Bender DA. Amino Acid Metabolism. Third Edition. Wiley-Blackwell, West Sussex. 2012.

Buse MG and Reid SS. Leucine. A possible regulator of protein turnover in muscle. Journal of Clinical Investigation, 56: 1250‒1261. 1975.

Cherkin A and Van Harreveld A. L-Proline and related compounds: correlation of structure, amnesic potency and anti-spreading depression potency. Brain Research, 156: 265‒273. 1978.

Cherkin A, Davis JL and Garman MW. D-Proline: stereospecificity and sodium chloride dependence of lethal convulsant activity in the chick. Pharmacology, Biochemistry and Behavior, 8: 623‒625. 1978.

Dancis J and Hutzler J. The metabolism of D- and L-pipecolic acid in the rabbit and rat. Biochimica et Biophysica Acta, 675: 411‒415. 1981.

D'Aniello G, Tolino A, D'Aniello A, Errico F, Fisher GH and Di Fiore MM. The role of D-aspartic acid and N-methyl-D-aspartic acid in the regulation of prolactin release. Endocrinology, 141: 3862‒3870. 2000.

D'Aniello S, Somorjai, Garcia-Fernàndez J, Topo E and D'Aniello A. D-Aspartic acid is a novel endogenous neurotransmitter. FASEB Journal, 25: 1014‒1027. 2011.

Dunlop DS, Neidle A, McHale D, Dunlop DM and Lajtha A. The presence of free D-aspartic acid in rodents and man. Biochemical and Biophysical Research Communications, 141: 27‒32. 1986.

Erwan E, Tomonaga S, Yoshida J, Nagasawa M, Ogino Y, Denbow DM and Furuse M. Central administration of L- and D-aspartate attenuates stress behaviors by social isolation and CRF in neonatal chicks. Amino Acids, 43: 1969‒1976. 2012.

Erwan E, Tomonaga S, Ohmori T, Mutaguchi Y, Ohshima T, Nagasawa M, Yasuo S, Tamura Y and Furuse M. Oral administration of D-aspartate, but not of L-aspartate, reduces food intake in chicks. Journal of Poultry Science, 50: 164‒171. 2013.

Erwan E, Chowdhury VS, Nagasawa M, Goda R, Otsuka T, Yasuo S and Furuse M. Oral administration of D-aspartate, but not L-aspartate, depresses rectal temperature and alters plasma metabolites in chicks. Life Sciences, 109: 65‒71. 2014a.

Erwan E, Chowdhury VS, Nagasawa M, Goda R, Otsuka T, Yasuo S and Furuse M. Central injection of L- and D-aspartate attenuates isolation induced stress behavior in chicks possibly through different mechanisms. European Journal of Pharmacology, 736: 138‒142. 2014b.

Furuse M and Okumura J. Nutritional and physiological characteristics in germ-free chickens. Comparative Biochemistry and Physiology Part A, Physiology, 109: 547‒556. 1994.

Grove JA and Roghair HG. The metabolism of D- and L-lysine in the chicken. Archives of Biochemistry and Biophysics, 144: 230‒236. 1971.

Gwee MC, Gopalakrishnakone P, Cheah LS and Bowman WC. L-Arginine-nitric oxide pathway involvement in the nerve-evoked relaxant responses of the 5-HT precontracted chick isolated upper oesophagus. Journal of Autonomic Pharmacology, 15: 151‒158. 1995.

Hamasu K, Shigemi K, Tsuneyoshi Y, Yamane H, Sato H, Denbow DM and Furuse M. Intracerebroventricular injection of L-proline and D-proline induces sedative and hypnotic effects by different mechanisms under an acute stressful condition in chicks. Amino Acids, 38: 57‒64. 2010.

Hashimoto A, Kumashiro S, Nishikawa T, Oka T, Takahashi K, Mito T, Takashima S, Doi N, Mizutani Y. Kaneco T and Ootomo E. Embryonic development and postnatal changes in free D-aspartate and D-serine in the human prefrontal cortex. Journal of Neurochemistry, 61: 348‒351. 1993a.

Hashimoto A, Nishikawa T, Oka T, Hayashi T and Takahashi K. Widespread distribution of free D-aspartate in rat periphery. FEBS Letters, 331: 4‒8. 1993b.

Hashimoto A, Oka T and Nishikawa T. Anatomical distribution and postnatal changes in endogenous free D-aspartate and D-serine in rat brain and periphery. European Journal of Neuroscience, 7: 1657‒1663. 1995.

Hutzler J and Dancis J. Conversion of lysine to saccharopine by human tissues. Biochimica et Biophysica Acta, 158: 62‒69. 1968.

Ishii C, Akita T, Mita M, Ide T and Hamase K. Development of an online two-dimensional high-performance liquid chromato-

graphic system in combination with tandem mass spectrometric detection for enantiomeric analysis of free amino acids in human physiological fluid. Journal of Chromatography A, 1570: 91−98. 2018.

Kanazawa T, Taneike I, Akaishi R, Yoshizawa F, Furuya N, Fujimura S and Kadowaki M. Amino acids and insulin control autophagic proteolysis through different signaling pathways in relation to mtor in isolated rat hepatocytes. Journal of Biological Chemistry, 279: 8452−8459. 2004.

Kawase T, Nagasawa M, Ikeda H, Yasuo Y, Koga Y and Furuse M. Gut microbiota of mice putatively modifies amino acid metabolism in the host brain. British Journal of Nutrition, 117: 775−783. 2017.

Kera Y, Aoyama H, Watanabe N and Yamada RH. Distribution of D-aspartate oxidase and free D-glutamate and D-aspartate in chicken and pigeon tissues. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 115: 121−126. 1996.

Kleckner NW and Dingledine R. Requirement for glycine in activation of NMDA-receptors expressed in Xenopus oocytes. Science, 241: 835−837. 1988.

Kurata K, Shigemi K, Tomonaga S, Aoki M, Morishita K, Denbow DM and Furuse M. L-Ornithine attenuates corticotropin-releasing factor-induced stress responses acting at GABAA receptors in neonatal chicks. Neuroscience, 172: 226−231. 2011.

Kurauchi I, Shigemi K, Yamada S, Aoki M, Kawada Y, Morishita K, Denbow DM and Furuse M. Comparison of central effects of L-ornithine metabolites on stress responses of neonatal chicks. Journal of Animal and Veterinary Advances, 8: 2580−2584. 2009.

Kurauchi I, Shigemi K, Kabuki Y, Hamasu K, Yamane H, Aoki M, Kawada Y, Morishita K, Denbow DM and Furuse M. Central L-ornithine, but not polyamines, induces a hypnotic effect in neonatal chicks under acute stress. Nutritional Neuroscience, 13: 17−20. 2010.

Kuzmicky DD, Kohler GO, Walker HG Jr and Mackey BE. Availability of oxidized sulfur amino acids for the growing chick. Poultry Science, 56: 1560−1565. 1977.

Maruyama K, Harper AE and Sunde ML. Effect of D-, DL- and D-glutamic acid on chicks. Journal of Nutrition, 105: 1012−1019. 1975.

Maruyama K, Sunde ML and Harper AE. Effect of D-alanine and D-aspartic acid on the chick. Journal of Nutrition, 102: 1441−1451. 1972.

Mordier S, Deval C, Bechet D, Tassa A and Ferrara M. Leucine limitation induces autophagy and activation of lysosome-dependent proteolysis in C2C12 myotubes through a mammalian target of rapamycin-independent signaling pathway. Journal of Biological Chemistry, 275: 29900−29906. 2000.

Morikawa A, Hamase H, Inoue T, Konno R, Niwa A and Zaitsu K. Determination of free D-aspartic acid, D-serine and D-alanine in the brain of mutant mice lacking D-amino acid oxidase activity. Journal of Chromatography B: Biomedical Sciences and Applications, 757: 119−125. 2001.

Morrison WD, Hamilton TS and Scott HM. Utilization of D-Tryptophan by the Chick. Journal of Nutrition, 60: 47−63. 1956.

Nagata Y, Horiike K and Maeda T. Distribution of free D-serine in vertebrate brains. Brain Research, 634: 291−295. 1994.

Nakano Y, Taniguchi M, Umakoshi Y, Watai D and Fukusaki E. High-throughput LC-MS/MS method for chiral amino acid analysis without derivatization. Methods in Molecular Biology, 2030: 253−261. 2019.

Nakashima K, Ishida A, Yamazaki M and Abe H. Leucine suppresses myofibrillar proteolysis by down-regulating ubiquitin-proteasome pathway in chick skeletal muscles. Biochemical and Biophysical Research Communications, 336: 660−666. 2005.

Nakashima K, Yakabe Y, Ishida A, Yamazaki M and Abe H. Suppression of myofibrillar proteolysis in chick skeletal muscles by alpha-ketoisocaproate. Amino Acids, 33: 499−503. 2007.

Neidle A and Dunlop DS. Developmental changes in free D-aspartic acid in the chicken embryo and in the neonatal rat. Life Sciences, 46: 1517−1522. 1990.

Newman RK and Sands DC. Dietary selection for lysine by the chick. Physiology & Behavior, 31: 13−19. 1983.

Nomura Y, Schmidt-Glenewinkel T and Giacobini E. In vitro formation of piperidine, cadaverine and pipecolic acid in chick and mouse brain during development. Developmental Neuroscience, 1: 239−249. 1978.

Ohara I and Ariyoshi S. Nutritive value of L-, DL- and D-tryptophan in the chick. Journal of Nutritional Science and Vitaminology, 25: 185−193. 1979.

Ohara I, Otsuka SI, Yugari Y and Ariyoshi S. Comparison of the nutritive values of L-, DL- and D-tryptophan in the rat and chick. Journal of Nutrition, 110: 634−640. 1980a.

Ohara I, Otsuka SI, Yugari Y and Ariyoshi S. Inversion of D-tryptophan to L-tryptophan and excretory patterns in the rat and chick. Journal of Nutrition, 110: 641−648. 1980b.

Otte DM, Barcena de Arellano ML, Bilkei-Gorzo A, Albayram O, Imbeault S, Jeung H, Alferink J and Zimmer A. Effects of chronic D-serine elevation on animal models of depression and anxiety-related behavior. PLoS One. 8: e67131. 2013.

Peyrollier K, Hajduch E, Blair AS, Hyde R and Hundal HS. L-leucine availability regulates phosphatidylinositol 3-kinase, p70 S6 kinase and glycogen synthase kinase-3 activity in L6 muscle cells: evidence for the involvement of the mammalian target of rapamycin (mTOR) pathway in the L-leucine-induced up-regulation of system A amino acid transport. Biochemical Journal, 350: 361−368. 2000.

Pico RM, Keller E, Cherkin A and Davis JL. Brain glutamate inhibition and amnesia: evidence provided by proline analog action. Brain Research, 285: 227−230. 1983.

Pullan LM, Olney JW, Price MT, Compton RP, Hood WF, Michel J and Monahan JB. Excitatory amino acid receptor potency and subclass specificity of sulfur-containing amino acids. Journal of Neurochemistry, 49: 1301−1307. 1987.

Reddy GK and Enwemeka CS. A simplified method for the analysis of hydroxyproline in biological tissues. Clinical Biochemistry, 29: 225−229. 1996.

Saito S, Tachibana T, Choi YH, Denbow DM and Furuse M. ICV CRF and isolation stress differentially enhance plasma corticosterone concentrations in layer- and meat-type neonatal chicks. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology, 141: 305−309. 2005.

Schell MJ, Cooper OB and Snyder SH. D-aspartate localizations imply neuronal and neuroendocrine roles. Proceedings of the National Academy of Sciences of the United States of America, 94: 2013−2018. 1997.

Scott ML, Nesheim MC and Young RJ. Proteins and amino acids. In: Nutrition of the chicken. 3rd ed. Pp. 58−118. M. L. Scotts & Associates, New York. 1982.

Shibuya N and Kimura H. Production of hydrogen sulfide from D-

cysteine and its therapeutic potential. Frontiers in Endocrinology, 4: 87. 2013.

Sugahara M, Morimoto T, Kobayashi T and Ariyoshi S. The nutritional value of D-amino acid in the chick nutrition. Agricultural and Biological Chemistry, 31: 77−84. 1967.

Suwandi L, Hausmann S, Braun A, Gruber T, Heinzmann SS, Gálvez EJC, Buck A, Legutko B, Israel A, Feuchtinger A, Haythorne E, Staiger H, Heni M, Häring HU, Schmitt-Kopplin P, Walch A, Cáceres CG, Tschöp MH, Rutter GA, Strowig T, Elsner M and Ussar S. Chronic D-serine supplementation impairs insulin secretion. Molecular Metabolism, 16: 191−202. 2018.

Takagi T, Ando R, Ohgushi A, Yamashita T, Dobashi E, Hussain-Yusuf H, Onodera R, Bungo T, Sato H and Furuse M. Intracerebroventricular injection of pipecolic acid inhibits food intake and induces sleeping-like behaviors in the neonatal chick. Neuroscience Letters, 310: 97−100. 2001.

Takagi T, Bungo T, Tachibana T, Saito E-S, Saito S, Yamasaki I, Tomonaga S, Denbow DM and Furuse M. Intracerebroventricular administration of GABA-A and GABA-B receptor antagonists attenuate feeding and sleeping-like behavior induced by L-pipecolic acid in the neonatal chicks. Journal of Neuroscience Research, 73: 270−275. 2003.

Tesseraud S, Bigot K and Taouis M. Amino acid availability regulates S6K1 and protein synthesis in avian insulin-insensitive QM7 myoblasts. FEBS Letters, 540: 176−180. 2003.

Tiedje KE, Stevens K, Barnes S and Weaver DF. β-Alanine as a small molecule neurotransmitter. Neurochemistry International, 57: 177−188. 2010.

Tischler ME, Desautels M and Goldberg AL. Does leucine, leucyltRNA, or some metabolite of leucine regulate protein synthesis and degradation in skeletal and cardiac muscle? Journal of Biological Chemistry, 257: 1613−1621. 1982.

Ungureanu-Longrois D, Bézie Y, Perret C and Laurent S. Effects of exogenous and endogenous nitric oxide on the contractile function of cultured chick embryo ventricular myocytes. Journal of Molecular and Cellular Cardiology, 29: 677−687. 1997.

Van Harreveld A, Cherkin A and Davis JL. Amnestic potency of proline analogs correlates with antispreading depression potency. Pharmacology, Biochemistry and Behavior, 12: 533−541. 1980

Wolosker H, Dumin E, Balan L and Foltyn VN. D-amino acids in the brain: D-serine in neurotransmission and neurodegeneration. FEBS Journal, 275: 3514−3526. 2008.

Yamane H, Asechi M, Tsuneyoshi Y, Denbow DM and Furuse M. Central L-cysteine induces sleep, and D-cysteine induces sleep and abnormal behavior during acute stress in neonatal chicks. Animal Science Journal, 80: 428−432. 2009a.

Yamane H, Tsuneyoshi Y, Denbow DM and Furuse M. N-Methyl-D-aspartate and alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate receptors involved in the induction of sedative effects under an acute stress in neonatal chicks. Amino Acids, 37: 733−739. 2009b.

Yoshida J, Erwan E, Chowdhury VS, Ogino Y, Shigemura A, Denbow DM and Furuse M. Comparison of centrally injected tryptophan-related substances inducing sedation in acute isolation stress-induced neonatal chicks. Pharmacology, Biochemistry and Behavior, 129: 1−6. 2015.

Zachar G, Jakó T, Vincze I, Wagner Z, Tábi T, Bálint E, Mezey S, Szökő É and Csillag A. Age-related and function-dependent regional alterations of free L- and D-aspartate in postembryonic chick brain. Acta Biologica Hungarica, 69: 1−15. 2018.

Zamenhof S and Ahmad G. The effects of exogenous nutrients on growth of chick embryo brain. Growth, 43: 160−166. 1979.