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Effects of the bioactive amino acids leucine and tryptophan on feed intake in layer chicks

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Abstract
Controlling feed intake is essential to providing ideal nutrition and helping poultry reach their maximum potential for growth and development. The current research looked at how L-leucine and L-tryptophan affected layer chicks' meal intake. Leucine and tryptophan were administered intracerebroventricularly (ICV) to 4-day-old layer chicks, and feed intake was monitored at different time intervals. Our findings demonstrated that L-leucine administration by ICV enhanced feed intake up to two hours after treatment (P 0.05). On the other hand, L-tryptophan (10 or 100 g) had no discernible impact on feed consumption. These results showed that L-leucine may impact appetite inside the hypothalamus and that orexigenic and anorexigenic Neuropeptide genes may be directly involved in these effects.

Keywords: L-leucine, L-tryptophan, intracerebroventricular (ICV), layer chicks, feed consumption

1. Introduction
Maintaining pullet health, laying hen quality egg output, and layer body development all depend heavily on nutrition (Wang *et al*., 2017) [43]. The nutrients or elements of animal food known as nutraceuticals have nutritional and pharmacological significance by protecting against different illnesses, having immunomodulatory potential, promoting good health, and thus boosting productivity (Dhama *et al*., 2015; Aronson, 2019; Helal *et al*., 2012; Waheed Janabi *et al*., 2020) [47, 3, 23, 41]. Amino acids, minerals, vitamins, fatty acids, enzymes, prebiotics, probiotics, symbiotics, pigments, medicinal herbs, herbal extracts, antioxidants, organic acids, flavouring agents, and other nutrients and non-nutrients are included in them (Narahari, 2014; Alagawany *et al*., 2018a; Elgeddawy *et al*., 2020) [33, 2, 48]. Due to the nutritional and physiological benefits of feed components as well as the negative consequences of chemical medicines, such as antibiotic resistance and drug residues, nutraceuticals have lately gained attention in poultry research (Elnesr *et al*. 2019a, 2020) [18]. Nutraceuticals may include vitamins, minerals, or a combination of them. They are often found in chicken rations (Ghoreyshi *et al*. 2019, Khatun *et al*. 2019, and Ahmad *et al*. 2019) [21, 27, 1], which are particularly significant in poultry nutrition. However, several important necessary amino acids (lysine, methionine, threonine, and tryptophan), vitamins, and minerals are often provided as synthetic supplements. Generally, poultry acquires nutrition from ingesting natural feedstuffs (Ravindran, 2021) [36]. Compared to traditional forms, refined dietary nutraceutical elements may improve digestion, absorption, utilisation, metabolism, and positive health impacts. To enhance bird production efficiency, numerous aspects should be considered, including the bird's genetic potential, environmental circumstances, food quality, and gastrointestinal health. These parameters include poultry's nutraceutical value and conversion efficiency (Rinttila & Apajalahti, 2018; Sugiharto, 2016; Yadav *et al*., 2019) [37, 39, 45]. This study aims to offer updates on the possible function of amino acids as bioactive compounds that will increase the productivity of layer chicks, safeguard the health and immunity of birds, and help with a variety of public health concerns.

1.1 Amino acids
Functional and structural components of protein and amino acids are divided into two categories by the food industry: non-essential (produced by the body) and essential amino acids (cannot be synthesised rapidly enough to meet the metabolic requirement). Amino acids are essential to the body's physiological processes (Bortoluzzi *et al*., 2018; Debnath *et al*.,

2019) [9, 14].

After absorption, amino acids are put together and converted into proteins, which are then utilised to construct various bodily tissues. According to studies, feeding pullets a high-protein, high-energy diet throughout their development and egg-laying phases favoured egg mass and yolk weight (Babiker *et al*., 2020) [5]. Different recommendations for the amounts of Necessary amino acids generate issues for the poultry industry because of these discrepancies. The use of synthetic amino acids in chicken feed has been well studied. The cautious use of synthetic amino acids may improve the overall amino acid balance and reduce the amount of crude protein in chickens' diets (Waldroup *et al*., 2018) [42].

1.2 The role of amino acids in the immune system

Birds' immune systems directly affect their health, and birds with healthy immune systems develop more quickly. The majority of necessary amino acids are thought to be vital nutrients for cytokine synthesis and immunological performance (Kidd, 2019; Li *et al*., 2017) [28, 31]. As a result, immunological stress or inflammation is likely to increase the need for critical amino acids (Le Floc'h *et al*., 2019) [49]. Additionally, amino acids are linked to the animal synthesis of antibodies (Han & Lee, 2018) [49]. In all species, proper immunocompetence and host defence against various illnesses depend on the intake of dietary amino acids (Becki *et al*., 2020) [6]. Therefore, if chickens consume enough amino acids in their diets, the development of immune function will be encouraged. According to research, birds perform better and have a stronger immune system when their food contains more threonine and methionine (Yaqoob & Ali, 2018) [46]. Immunoglobulins include threonine, which functions as a proteinogenic amino acid. Increased threonine intake may boost the development of immune cells, encourage the production of antibodies, and lessen the immunological stress brought on by the Newcastle disease (ND) virus or Escherichia coli challenge (Azzam El-Gogary, 2015; Trevisi *et al*., 2018) [4, 40]. Additionally, according to Bhanja *et al*. (2016), layers fed a diet containing 1.02% threonine had 17% greater bursa weight, 7% greater thymus weight, and 16% greater spleen weight compared to layers fed a control diet containing 0.96% of threonine. Tryptophan, used as a supplement due to its requirement for protein synthesis, also functions as a precursor of serotonin, a neurotrans

1.3 Amino acid absorption and transportation

Amino acids and peptides are absorbed in the epithelial cell's brush edge. Enterocytes take in amino acids as free amino acids, dipeptides, and tripeptides. The jejunum and ileum are the body parts where amino acid and peptide absorption happens most quickly. Transport is carried out by peptide and amino acid transporters found on the membrane of enterocytes. There are three main transporter systems, according to Broer (2018) [9]: the neutral system, the basic system, and the acidic system.

1.4 Leucine

Leucine, an essential amino acid, probably serves as a physiological indicator of the availability of amino acids in the hypothalamus (Blouet *et al*., 2019) [8]. It is the most powerful stimulator of the mammalian amino-acid-sensitive mTORC1 pathway and reaches the brain more rapidly than other amino acids (Blouet *et al*., 2019; Proud, 2019) [8]. By boosting hypothalamic m TOR signalling, leucine intracerebroventricular (ICV) injection decreased animal food intake (Cota *et al*., 2016) [12]. Additionally, it was shown that feeding pigs a diet low in valine but high in leucine caused a rapid decline in feed consumption (Gloaguen *et al*., 2021) [22]. On the other hand, leucine greatly increased food intake in newborn chicks when it was given centrally (Izumi *et al*., 2019) [26]. Leucine was injected intravenously, although the impact on hypothalamic orexigenic and anorexigenic Neuropeptide was still unclear.

1.5 Tryptophan

In laying hens, tryptophan is an important amino acid for protein synthesis and several other metabolic functions. Although necessary, it is still being determined from the literature whether trp is the third or fourth limiting amino acid for laying hens. There has been little investigation on the trp requirement in laying hens (Ishibashi, 2020; Peganova & Eder, 2021) [25, 34]. As a crucial necessary amino acid, trp contributes to the synthesis of body proteins and the generation of protein to assist the formation of eggs (Wu, 2019) [1]. TRP participates in several metabolic activities in addition to protein synthesis. Dietary Trp is linked to mood, stress response, sleep, and appetite management since it is a precursor to serotonin, a crucial neuromediator (Le Floc'h & Seve, 2017) [50]. Tryptophan is also involved in the metabolism of nicotinamide adenine dinucleotide (NAD+), niacin, and picolinic acid via the Kynurenine pathway.

Reduced appetite and feed intake were the major effects of tryptophan deprivation on animal development (Eder *et al*., 2019; Le Floc'h & Seve, 2017) [50, 29]. In contrast to central administration, which had been demonstrated to reduce food intake in free-fed chicks, dietary tryptophan dramatically increased body weight growth and feed consumption in chicken (Emadi *et al*., 2021) [20]. (Bungo *et al*., 2018) [10]. However, the effects of tryptophan central injection on hypothalamic orexigenic and anorexigenic Neuropeptide in layer chicks have yet to receive much study attention.

2. Materials and Methods

2.1 Experimental animals

Layer chicks aged one day were bought from a hatchery and kept in a room with a constant temperature of 30 1 °C. Every day, lighting was delivered nonstop for 24 hours. Free access to water and a commercial beginning feed was provided for the chicks. The chicks (3-d old) were chosen and divided into 12 experimental groups based on their body weight and average feed intake 1 day before the experimental day, ensuring that the average body weight was as uniform as feasible within the same experimental group.

2.2 Preparation of drugs

L-leucine and L-tryptophan were then dissolved in 0.85% saline, including 0.1% Evans Blue solution to aid injection site localisation, for a total injection volume of 5 L. We chose two dosages of each amino acid (0.15 or 1.5 mol for L-leucine and 10 or 100 g for L-tryptophan) based on similar/other tests conducted on chicks by other researchers (Izumi *et al*., 2019; Bungo *et al*., 2018; Suenaga *et al*., 2018) [26, 38, 10].

2.3 Intra-cerebroventricular (ICV) injection procedure

The technique used to inject the chicks was modified by Davis *et al*. (2019) [13] and Cline *et al*. (2018) [11]. After data collection, the chick's skull was sectioned along the frontal plane to locate the injection location. The lateral ventricle system of any chicken lacking dye was removed before the examination. Feeding intake studies 144 4-day-old layer chicks were given L-leucine (0.15 mol or 1.5 mol in vehicle), L-glutamate (0.86 mol or 1.6 mol in vehicle), L-tryptophan (10 mol or 100 mol in vehicle), L-arginine (20 mol or 200 mol), or vehicle control (0.85% saline containing 0.1% Evans

Blue in the volume of 5 L) by ICV administration. The chicks were given an injection and then placed back in their separate cages with free access to food and water. After administration, feed intake was observed at the following intervals: 0.25, 0.5, 1, 1.5, and 2 hours. We weighed the remaining diet at different time points (0.25, 0.5, 1, 1.5, and 2 h) so that we could calculate the cumulative feed intake of various time points after giving chicks a specific amount of diet in a cup at the start of the experiment (0 h).

3. Results and Discussion

1. Results ICV injection of leucine increased feed intake

Leucine injections of 0.15 mol and 1.5 mol were given to see how they affected the animals' meal consumption. As seen in Figure 1, the 0.15 mol group's feed intake increased significantly (P 0.05) at 0.5, 1, 1.5, and 2 h after injection. In comparison, the 1.5 mol group's feed intake increased significantly (P = 0.052) at 1 and 2 h after ICV injection. At 1.5 h after injection, feed intake was very close to a significant increase (P = 0.052). The 1.5 mol feed intake rose 0.5 h after injection; however, this rise was not statistically significant. As a result, leucine at both doses had a noticeable stimulatory impact on feed intake, with 0.15 mol having a substantially stronger effect up to 2 h after injection.

Table 1: Results ICV injection of leucine increased feed intake

At 0.25h		At 0.5h		At 1h		At 1.5h		At 2h	
Leucine	**Feed Intake (g)**	**Leucine**	**Feed Intake (g)**	**Leucine**	**Feed Intake (g)**	**Leucine**	**Feed Intake (g)**	**Leucine**	**Feed Intake (g)**
C	1.0	C	1.25	C	1.50	C	1.75	C	2.0
0.15µmol	1.5	0.15µmol	1.75	0.15µmol	2.0	0.15µmol	2.25	0.15µmol	2.50
1.5 µmol	1.25	1.5 µmol	1.50	1.5 µmol	1.75	1.5 µmol	2.00	1.5 µmol	2.25

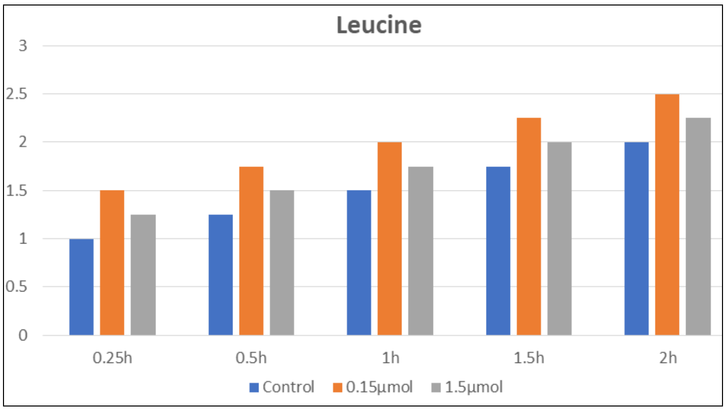

Fig 1: ICV injection of leucine increased feed intake

2. Tryptophan ICV injection had no significant effect on food intake in layer chicks

To determine how tryptophan ICV injection affected layer chick feed intake, 10 and 100 g of L-tryptophan were given. Even at a greater dosage (100 g), there was no discernible change in feed intake compared to the control group. Compared to the control, the 10 g L-tryptophan dosage slightly decreased feed intake at 0.25 h, 1 h, and 1.5 h post-injection (P = 0.189, 0.224, and 0.270, respectively) over the 2-hour post-injection period. The small feed intake decrease was uniform and constant even though it was not large. Figure 2 displays the findings in summary.

Table 2: Tryptophan ICV injection had no significant effect on food intake in layer chicks

At 0.25h		At 0.5h		At 1h		At 1.5h		At 2h	
Tryptophan	**Feed Intake (g)**	**Tryptophan**	**Feed Intake (g)**	**Tryptophan**	**Feed Intake (g)**	**Tryptophan**	**Feed Intake (g)**	**Tryptophan**	**Feed Intake (g)**
C	1.6	C	1.8	C	2.0	C	2.2	C	2.4
10 µg	1.5	0.15µmol	1.7	0.15µmol	1.9	0.15µmol	2.1	0.15µmol	2.2
100 µg	1.6	1.5 µmol	1.8	1.5 µmol	2.0	1.5 µmol	2.2	1.5 µmol	2.3

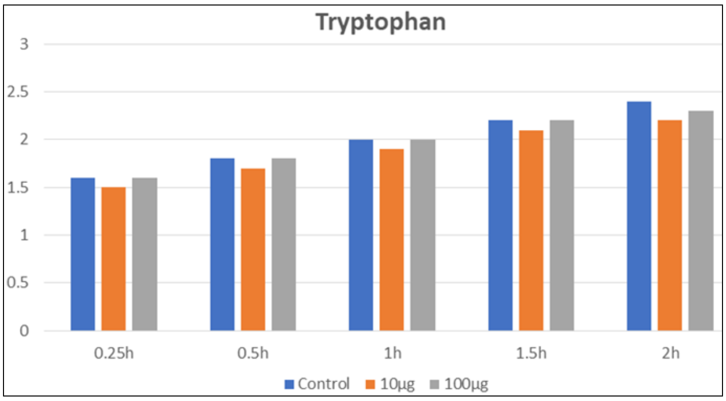

Fig 2: Food intake in layer chicks was unaffected significantly by tryptophan ICV injection

4. Discussion

The key finding from the current data was that leucine injection by ICV promotes feed intake in layer chicks. For the lesser dosage (0.15 mol) of leucine, feed intake was noticeable at 0.5 hours after injection and persisted for 2 hours. The greater leucine dosage significantly increased feed intake from 1 hour after injection and maintained it for 2 hours after injection. Therefore, the smaller dosage was more successful in affecting feed consumption.

According to the study, tryptophan ICV injection had no appreciable impact on the layer chicks' eating ability after a three-hour fast. This was true even though the 10 g dosage caused a little reduction in feed consumption that persisted for 2 h after injection. The 100 g dosage of L-tryptophan may have been too large compared to the 10 g dose to impact feed consumption substantially. A study by Bungo *et al*. (2018) [10] demonstrated that L-tryptophan ICV injection of 3-day-old chicks fed ad libitum significantly reduced feed intake, indicating that tryptophan injected into chicks' brains was quickly converted to serotonin (5-hydroxytryptamine, or 5-HT), which in turn induced hypophagia via the 5-HT2A receptors (Bungo *et al*., 2018) [10]. Thus, more research into the impacts of central tryptophan on food intake in layer chicks under various physiological circumstances was still required.

5. Conclusion

According to the findings of this research, L-leucine and L-glutamate may function inside the hypothalamus to affect how much food is consumed, and orexigenic and anorexigenic Neuropeptides may directly impact this Effect. Leucine infusion into the ICV boosted feed consumption. When given intravenously to layer chicks, tryptophan may not directly impact meal consumption**.**

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