

# ROLE OF TRYPTOPHAN ON POULTRY PERFORMANCE, BEHAVIOUR, AND WELFARE

## ABSTRACT

The genetic potential of poultry has advanced exponentially in the past 50 years. The current broiler, turkey, and laying hens are responsive to better nutritional balance. The amino acids play a huge role in maintaining the growing protein requirement of modern birds for meat deposition, physiological needs, health status, immune system, and combating behavioural problems. Apart from protein synthesis, many amino acids play a substantial functional role in animals. One of these amino acids is Tryptophan. Tryptophan is an essential amino acid that should be supplemented through diets. Tryptophan plays an important

role in protein synthesis and serves as a precursor for crucial neurotransmitters, such as serotonin and melatonin. It is involved in physiological growth, immune response, behaviour, stress mitigation, and appetite control. Tryptophan can be an important link for welfare through nutrition in modern poultry. Therefore, this review will cover the main functional roles of tryptophan in broilers, laying hens, and turkeys to provide producers and nutritionists with an important tool in their nutritional toolbox to address production and welfare issues through nutrition.

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**Keyword** Amino acids, Behavior, Poultry, Tryptophan, Welfare



## BACKGROUND

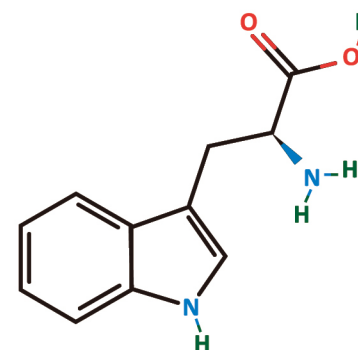
Poultry genetic and nutritional advancement in the past 50 years has been exponential. The strength and development of the genetic potential and nutrition, disease, and management balance have resulted in a cheaper, more nutritious, and easily accessible protein source as poultry meat and eggs for human consumption. The growing population of the world is targeted to reach 9.8 billion by 2025 and 11.2 billion by 2100, with about 83 million people being added to the world's population every year (UN, 2017). It is even more important to supply the world's growing population with a healthy, safe, and affordable protein source. Poultry meat and eggs are some of the best natural protein sources to meet the growing demand. However, the poultry industry has its own struggles to meet the fine demand of cost-effective production pressure, environmental and nutritional management, and welfare of the growing meat and egg birds. The growth projection, production parameters, and nutritional requirements of modern broilers, laying hens, and turkeys have changed rapidly in the last 25 years. Modern broilers and turkeys are growing bigger with better FCR and profitability with delicately nutritionally balanced feed, meeting their genetic potential.

The amino acids play a huge role in maintaining the growing protein requirement of the birds for meat deposition, physiological needs, health status, and behavioural problems mitigation. Laying hens have also genetically advanced over the years. Now, the birds can have a long lay cycle of 100 weeks while producing about one egg daily. This demands a huge nutritional balance and production management in laying hens. As the genetics and housing systems have evolved, other problems with laying hens have also been emerging such as feather pecking, nervousness, and piling mortality. Such behavioural problems can be mitigated with management and nutritional intervention. One of the most important components of nutrition is to balance the amino acids in the diet to meet its rigorous nutritional demand. Breeders are even delicate on the need for a fine balance of nutrition and management. The challenge is to have the best laying and fertile bird possible without overweight birds while maintaining the nutritional and welfare needs of the birds. Currently, the birds are fed a restricted crude protein (CP), and energy diet. Lysine (Lys) and methionine (Met) are balanced, while other lower-limiting amino acids are mostly overlooked and let it float based on the ingredients used. However, the requirement of these lower-limiting amino acids, such as Tryptophan (Trp), Threonine (Thr),

Arginine (Arg), and the Branch chain amino acids (BCAA) must be met to provide the physiological requirement of the body and egg production, and to improve fertility and hatchability of eggs.

Nutrition plays a significant role in supporting the genetic potential of poultry. The birds are growing faster and require supplementation of precise nutrition for best performance, welfare, and profitability. Tryptophan is one of the essential limiting amino acids for poultry, so an external dietary source should supply it. The analysis of meat and plasma may result in the lowest proportion of Trp compared to other amino acids. However, as the research has uncovered, there are other more prominent physiological and functional roles of Trp in poultry rather than just the needs for protein deposition. Trp is the precursor for serotonin, kynurenine, and niacin synthesis [Figure 1](#), affecting behaviour, gut health, and brain-gut cross-talk (Le Floch et al., 2011). Serotonin is an important neuromodulator that regulates gastrointestinal function, mood, appetite, and hemodynamics. The lower proportion of Trp available through current commercial diets and higher demand of Trp for physiological roles can create marginal deficiency resulting in behavioural problems and drag optimum protein deposition. If Trp is not adequately supplied or has antagonism in absorption and metabolism, it increases the risk of unbalanced homeostasis.

**Figure 1** Tryptophan chemical structure

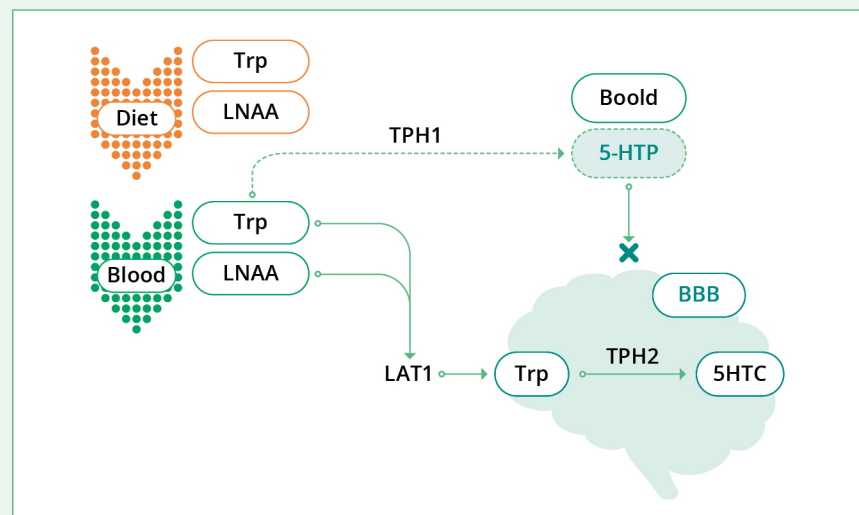


Tryptophan can be the fourth or fifth limiting amino acid in breeders. Breeder diets are usually fed low in CP, in which lower levels of Lys are balanced with macro protein ingredients and restricted feed intake. This makes Trp levels even lower in breeder diets, making them susceptible to behavioural problems like feather pecking, feather licking, and cannibalism. Crystalline Trp [Figure 1](#) can help in such scenarios by increasing the Trp levels in the diet without increasing the CP level. In laying hens, Trp can be 4th or 5th limiting amino acid. Apart from just the need for production and physiological needs, laying hens that are raised in enriched colonies, free range, or cage-free environments will need more Trp in the diet to support behavioural problems such as cannibalism, feather pecking, and piling mortality. In turkeys, Trp can be 4th or 5th limiting amino acid in the grower and early finisher diets. If the Trp demand is not met in those diets, there can be behavioural problems in turkeys, such as aggressive picking, cannibalism, and scratches in the carcass leading to condemnation.

Apart from essential amino acids, Trp is part of the large neutral amino acids (LNAAs) along with Valine(Val), Isoleucine (Ile), Leucine (Leu), Phenylalanine (Phe) and Tyrosine (Tyr). If the diets have higher neutral amino acids such as Leu or Phe, that can increase the large neutral amino acid pool compared to Trp, creating a situation where Leu and Phe compete with Trp for absorption. This antagonism can cause lower absorption and availability of Trp in the animal. Antagonism is common in high corn or corn byproduct (DDGS, corn gluten meal) based diets, which are common in the American and Asian continents. Higher corn and corn byproduct diets also contain relatively lower levels of Trp compared to BCAA, which can exacerbate the problems. Laying hen diets already contain high amounts of corn in the diet and the addition of more DDGS or corn gluten to replace soybean meal (SBM) can increase the potential SID Leu:Lys in the diet to above 170-200, impairing the availability and absorption of Trp in laying hens and breeders. Moreover, Trp metabolite 5-HT that is formed in the peripheral blood cannot cross the blood-brain barrier. Thus, Trp have to cross the blood-brain barrier and form the 5HT in the brain to improve the behavioural pattern. If the pool of Trp is low or is competing with LNAA to cross the blood brain barrier, there will be limited Trp in the brain causing even more stress for birds (Oh et al., 2015) [Figure 2](#). A table with different levels of Leu, Phe, and Trp in proportion to CP and Lys is provided [Table 1](#).

In a normal broiler and Turkey feed, the Leu:Lys ratios are about 145, Leu:Lys ratios in laying hens are 165, and Leu:Lys ratios in breeder feed are above 200. Such high Leu can cause

**Figure 2** Source of dietary tryptophan (Trp) and large neutral amino acids (LNAA) flow inside the body through blood



Adapted from: Spiegelhaar and Warma, 2023. DOI: 10.5772/intechopen.1004045

a reduction of Trp absorption in the gastrointestinal track and blood brain barrier, thus demanding a higher Trp supply in the diet.

In the past, the poultry industry used to rely on the intact protein source to fulfil the Trp requirement for the birds. If the nutritionist wanted to increase the Trp ratio by 2 or 3 points in the diet, the only way was to increase the protein source such as SBM or meat bone meal (MBM) or other locally available protein ingredients. However, in doing so, the crude protein of the diet would increase so high that it may, in turn, create more problems in birds than the intended positive impact. However, with technological advancement, feed-grade Trp is available in a cost-effective form that can increase the Trp level in the diet without increasing the CP to detrimental levels. Trp can be an integral tool in the toolbox of nutritionists to tackle the behavioural problems in poultry and balance numerous physiological requirements of the birds through nutrition. This review will summarize some of the research publications on the benefits of Trp in poultry in different situations and production parameters.

**Table 1** Amino acid (Lysine, Tryptophan, Isoleucine, Leucine, Valine, and Phenylalanine) composition of a few common ingredients used in poultry feed formulation +

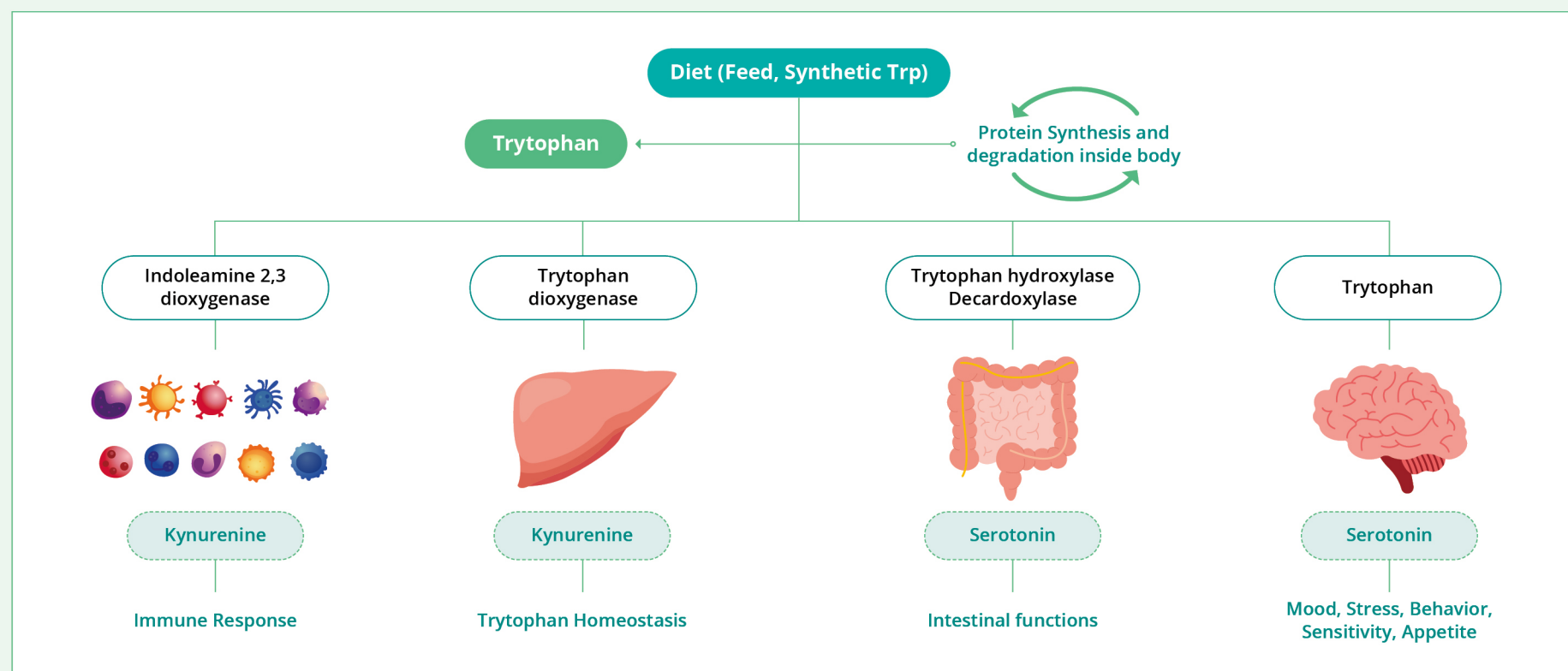
Ingredients / % total	LYS	TRP	ILE	LEU	PHE
Corn	0.22	0.06	0.26	0.74	0.33
DDGS	0.63	0.23	0.96	3.11	1.26
SBM	2.82	0.61	1.85	3.31	2.14
MBM	2.17	0.21	1.40	2.80	1.50
Milo	0.20	0.10	0.34	1.12	0.46
Hominy meal	0.39	0.09	0.30	0.86	0.40
Red wheat W	0.37	0.18	0.58	0.94	0.71
Bakery meal	0.30	0.10	0.36	0.80	0.40
Corn gluten meal	1.00	0.25	2.29	10.11	3.77
Peanut meal	1.54	0.48	1.55	2.97	2.41
Canola meal	2.45	1.51	2.65	1.94	1.52



### Metabolism of Tryptophan

Tryptophan is an essential amino acid and is an important precursor in many physiological needs of poultry. The first use of Trp is in protein synthesis. The average Trp needed for protein content per 100 gm of protein is 1.2g, which is much lower than for Lys (7.6), Leu (7.1), and Thr (4g) (Mahan and Shields, 1998). This requirement of Trp for protein formation can be deceiving while running the Trp requirement study based solely on the performance of the animals. Apart from just performance and protein synthesis, another more important role of Trp is the physiological regulation of various pathways and enzymes in the body **Figure 3**. Tryptophan is the precursor of serotonin (5-HT or 5-Hydroxytryptamine), an important neuromodulator regulating mood, appetite, and gastrointestinal functions. Conversion of serotonin occurs in two steps: 1. Conversion of Trp into 5-hydroxytryptophan by tryptophan hydroxylase (TPH). TPH1 is found in enterochromaffin, and THP2 is found in neurons. 2. Decarboxylation of 5-hydroxytryptophan into serotonin. This last step is vitamin B6 dependent.

**Figure 3** A simple graphical description of role of Tryptophan function and metabolism within different tissue



(Source adapted from Folc'h et al., 2010).

Tryptophan is also the precursor of N-formalkynureine. N-formalkynureine is converted to kynurenine by kynurenine formamidase. These are the first metabolites of the metabolic pathway that form niacin, kynurenine, and xanthurenic acid. In both the peripheral and central nervous systems, the kynurenine pathway (KP) represents the major route for the catabolism of L-Trp, resulting in the production of the essential co-factor pyridine nucleotide nicotinamide adenine dinucleotide (NAD+) and other neuroactive intermediates (Jones et al., 2013)

Tryptophan is also utilized and incorporated into constitutive and exported proteins such as acute phase proteins, C-reactive proteins, haptoglobin, and fibrinogen during inflammatory challenges. Preston et al. (1998) suggested that in cancer patients suffering from an inflammatory response, Trp would be the limiting amino acid for fibrinogen synthesis.

Metabolism and the function of Trp in the brain are similar but unique due to the role of the blood-brain barrier. Tryptophan is transported to the brain by transporters loaded in the capillaries of the blood-brain barrier, which are also shared by large neutral amino acids. The proportion of large neutral amino acids such as Leu, Val, Ile, tyrosine, methionine, and phenylalanine to Trp can influence the amount of Trp entry in the brain. Tryptophan hydroxylase, the rate-limiting enzyme of serotonin synthesis, is not saturated in the brain's Trp concentration; thus, the amount of Trp transported into the brain could be directly related to the amount of serotonin synthesis. Thus, increasing dietary and plasma Trp can have a direct relationship to the brain serotonin, and experimental acute Trp depletion in the diet causes the decrease of Trp and serotonin in the brain.

### **Tryptophan in laying hens**

Welfare related to feather pecking and cannibalism in different housing systems is a major behavioural problem in laying hens. The laying hen industry housing system is rapidly evolving and consists of a range of housing systems in different countries. Some of the housing systems consist of caged housing, where three to four laying hens are kept per pen; enriched colony housing, where 30-100 laying hens are kept

per pen; cage-free housing and free range, where the birds are free to roam in the floor and different tiers within or outside the house. These changes in housing systems are rapidly evolving in developed countries like Europe, Australia, and the USA. As the genetics and housing system have evolved, laying hen feathering and welfare are becoming important topics in the laying hen industry, with feather pecking, mortality, and a decrease in egg production being major issues with behaviour problems if they escalate. Excessive feather pecking can lead to cannibalism, skin damage, mortality which negatively impacts health and production.

Utilizing nutritional interventions such as Trp can play a significant role in alleviating the welfare problems in laying hens in different housing systems and stages of life. Numerous studies have been conducted on laying hens to understand the positive benefits of using Trp for improving behavioural problems, egg production, and the health of laying hens. Trp acts as a precursor to serotonin and melatonin, and exhibits antioxidant properties (Wu, 2009; Dong et al., 2017; Yue et al., 2017). Serotonin and melatonin regulate and decrease aggression, alleviate stress levels, and enhance adaptability to social environments (Martin et al., 2000; Le Floch and Seve, 2007).

Wen et al. (2019) conducted an experiment in small-framed first-cycle laying hen from 41 to 60 wks of age. The experiment fed six Trp:Lys ratio from 10% in the basal diet to 22.5% in the summit diet with the addition of L-Trp. The lowest concentration of Trp resulted in reduced egg production and was terminated from the experiment by 45 weeks. This shows that lower Trp can also hamper egg production. Plasma serotonin responded linearly to the Trp concentration. The addition of Trp also responded with better hen house egg production, egg mass, and feed conversion ratio. Dose-response on laying hen performance (Hen house egg production, egg weight, egg mass) to increasing Trp to a certain level has been shown in numerous studies (Bray, 1969; Russell and Harms, 1999; Bregendahl et al., 2008; Wen et al., 2019). Wen et al. (2019) also reported that there could be a decrease in the feed intake of birds if the Trp concentration falls below 0.156 in the diet. Several other studies have reported reduced feed intake with low dietary Trp concentration (Bray, 1969; Jensen et al., 1990; Russell and Harms, 1999). This is more important for laying hens raised in tropical conditions where the temperature can already lead to lower feed intake. Lower Trp levels in the diet can lead to a further

decrease in egg production and feed intake. Feeding the Trp at or more than 0.176% (Trp: Lys ratio of 20) increased yolk weight in the experiment (Wen et al., 2019). Similarly, another study found that the dig Trp: Lys ratio of 24.5% in the feed of white laying hens in the production stage promotes better animal performance and histological results (Lima et al., 2012).

The increasing dietary concentration of Trp in the laying hen diets increases serotonin, which can also be a good mitigation to behavioural problems. Serotonin is a neurotransmitter that influences the occurrence and regulation of abnormal behaviour (van Hierden et al., 2004). Increasing Trp in the diet above the Trp: Lys ratio of 20 significantly increased serotonin concentration in the blood plasma of laying hens (Laycock and Ball, 1990; Wen et al., 2019). In another study from Son et al. (2025), increasing the Trp concentration in the diet increased serotonin concentration. Trp is known as a precursor to serotonin synthesis, which regulates the stress response in the body and impacts the corticosteroids secreted from the hypothalamic-pituitary-adrenal axis (Fuller, 1995). A series of experiments was conducted to understand the effect of acute Trp depletion in the diet on the feather peaking of birds (Birkel et al., 2019a; b). The experiment showed that acute Trp depletion increased the light and severe repetitive feather peaking in both high and low feather pecking lines of birds. In a study with two genetic lines, one selected for low mortality due to injurious pecking and another for high production line from 22 to 29 wks of age, it was found that head/comb injuries inflicted via aggressive pecking increased significantly with lower Trp availability and relatively higher large neutral amino acids in plasma of those birds. This indicates that lower Trp:LNAAs in the plasma can trigger behaviour response in laying hen (Birkel et al., 2017). Thus, it is essential to notice the Leu level in the diet of laying hens, which is high in Corn, DDGS, and Corn Gluten meal. These macro ingredients can increase the Leu level in the diet while being low in Trp, exacerbating the Trp deficiency in the diet and poor absorption of Trp in gastrointestinal track of laying hen. Hierden et al. (2004a) showed that a reduction of 5-HT neurotransmitter in brain increases the severe feather pecking behaviour in both high and low feather pecking line laying hens. In a follow up experiment high and low feather pecking line of laying hens were fed either diet containing either 0.16% Trp or 2% Trp. After 14 days of treatment period Trp fed birds had significant reduction in gentle and severe feather pecking behaviour. Birds fed

higher Trp had significantly higher Trp/LNAA ratio in plasma and significantly increased %HT turnover in hippocampus and archistriatum and tended to do so in the remainder of the fore brain (Hierden et al., 2004b).

Besides altered monoamine neurotransmitter systems, dysregulation of the gut microbiome (van der Eijk et al., 2019b) and immune system (van der Eijk et al., 2019a), and hyperactivation of the hypothalamic-pituitary-adrenal axis (HPA) (Kjaer and Guémené, 2009)) have been associated with feather peaking behaviour in laying hens. 5-HT, which acts at the microbiota-gut-brain axis, can modulate the related network (Gheorghe et al., 2019; Huang et al., 2023). In another study with a laying hens at 28 weeks of age, supplementing Trp increased glutathione peroxidase, total superoxide dismutase activity, and total antioxidative capacity concentration and decreased malondialdehyde concentration. Serum IgA concentration increased and serum IgM increased linearly while laying hens were fed a dietary Trp concentration of 0.21-0.23% (Dong and Zou, 2017).

Thus, Trp concentration in the diet, availability of Trp in laying hen through gastrointestinal tract and getting the Trp from peripheral blood to pass the blood brain barrier is important to get the behavioural benefits in laying hens. Understanding the digestive dynamics of LNAA and Trp in the gastrointestinal tract and concentration of LNAA and Trp in the feed ingredients that are used in laying hen diets are important to balance to reduce the chance of Trp deficiency in laying hens.

### **Tryptophan in Breeders**

As the broilers are bred to be more efficient in meat production and improve in FCR and efficiency, it is increasingly difficult to manage breeders as they crave dense nutrition like broilers are highly responsive to nutritional tweaks. However, to get the best egg production, hatchability, and fertility, the breeders are managed to a specific weight according to age with proper nutritional balance. In such an instance, there is a high chance that Trp along with lower limiting amino acids are never even observed in the breeder diets. This lower Trp, high Leu in the diet, and higher metabolic and physiological demand of breeders create a complex Trp deficiency, leading to behavioural problems.



Decreases in hatchability, fertility, and egg production, together with feather licking, feather pecking, and hens with no feathers, are some of the increasing problems in breeders. Decrease in egg production, egg weight, egg mass, fertility, and hatchability percentage have been recorded in broiler breeder hens fed Trp-deficient diets (Russell and Harms, 1999; Jiang et al., 2018). Female broiler breeders are managed to reach the weight of about 7 lbs at 41 weeks of age. Roosters are managed to reach a similar weight of 7lbs at 23 weeks of age [Figure 4](#). The broiler from the egg of the breeder reaches the same age at about 7 weeks of age (Aviagen, 2022). The growth capacity of the broiler breeder is similar to its progeny. However, to maintain weight, fertility, and reproductive function, broiler breeders are fed a finely curated diet to meet their nutrition and funding needs with low Lys and low CP diets. However, if all the lower limiting amino acids are just based on feeding low CP, and Lys are only met through intact protein sources from feedstuffs, functional amino acids such as Met, Trp, and Arg can be limiting in the birds that can increase the aggressive behaviour, increase aggression during the feeding period, decrease clean up time, decrease uniformity, and poor feathering in the flock. All of this leads to reduced egg production, fertility and hatch, and an increase in aggressive behaviour (feather pecking and licking) and cannibalism in breeders. Supplementing Trp in the diet in crystalline form can increase the Trp level in the diet without increasing the Lys or CP in the diet, thus maintaining the requirement of birds but giving a boost of the Trp to alleviate the behaviour issues.

Numerous studies have been conducted and published to show the positive benefits of Trp in breeders and laying hens in terms of egg production, fertility, and aggressive behaviour, such as feather pecking and feather licking. A study by Jiang et al. (2018) conducted with yellow-feathered broiler breeders indicated that feeding graded levels of Trp in the diet at 0.11%, 0.15%, 0.19%, 0.23%, and 0.27% produced a quadratic response to laying rate, egg production, total large follicle weight, and average large follicle weight. The hatchability and fertilization rate of the total eggs were increased in the breeders fed 0.23 and 0.27% Trp compared to the breeders fed with the 0.19% Trp (Jiang et al., 2018). Research has indicated that an appropriate level of Trp is required in the diet for appropriate ovalbumin production (Kim and Choi, 2014). Lower levels of Trp can inhibit the low-density lipoprotein in the life and inhibit the follicle weight, thus affecting fertility and egg weight (Rogers and Pesti, 1992; Ruan et al., 2019). In a broiler breeder experiment where the birds were fed a basal diet with the lowest level of Val, Ile, and Trp with various levels of increased Val, Ile, and Trp in the diets.

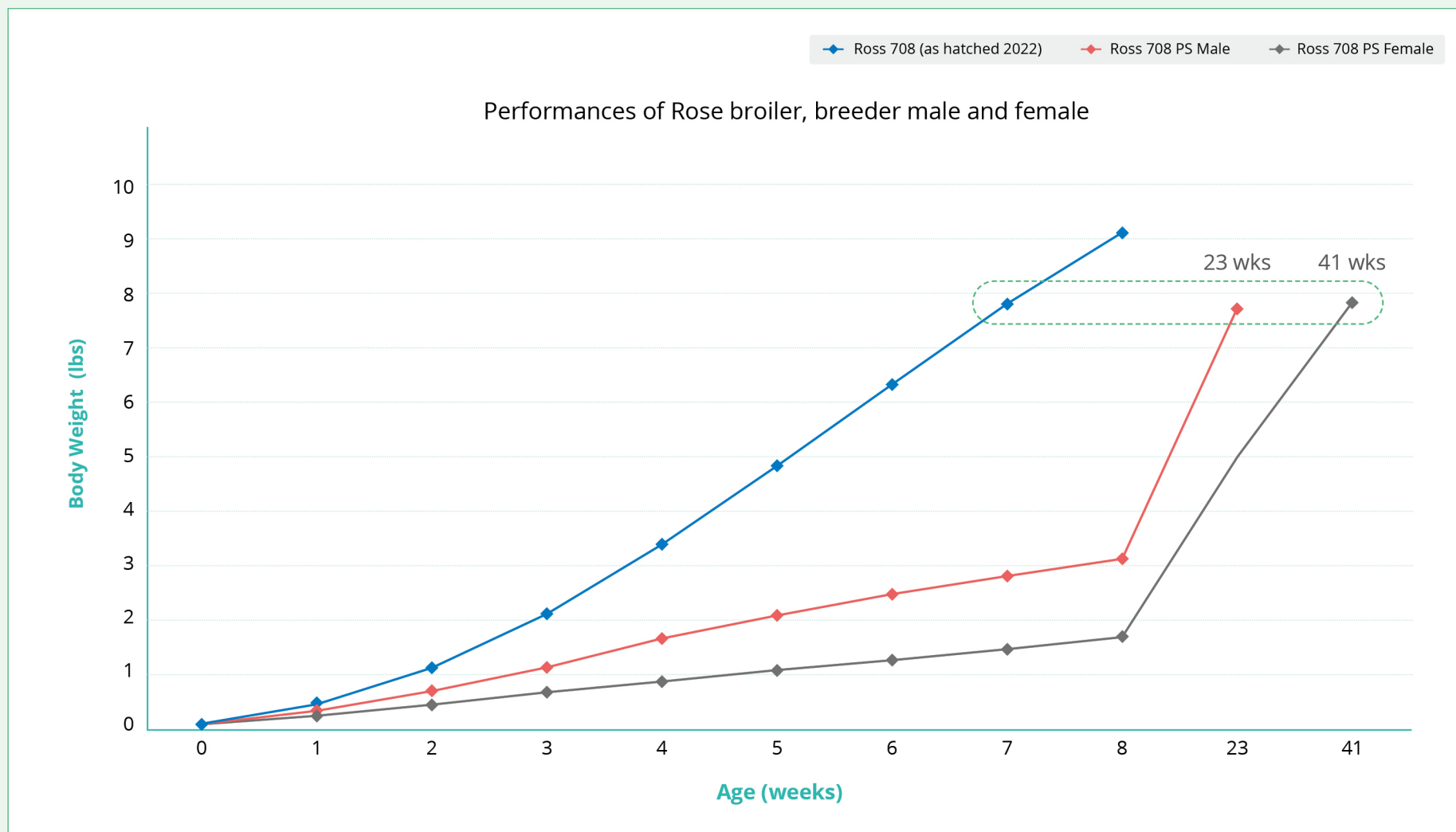
Increased Trp in the diets improved feed intake, rate of lay, egg weight, and egg output (Lima et al., 2018).

Two experiments were conducted on male broiler breeders to understand the effect of supplying Trp in aggressive behaviour. Three 20-minute observations were done per week to record the behaviour pattern. The addition of Trp in both experiments significantly ( $P < 0.05$ ) decreased pecking and threatening behaviour in both developing and mature broiler breeder males (Shea et al., 1990). A study was conducted to evaluate the effects of five inclusion levels of dietary Trp on growth and fear response from both types of broiler breeders (Ross and Cobb). The inclusion of L-Trp had a shorter latency to the right and spent less time in the first (immobile) phase of tonic immobility compared to the birds fed basal diet, indicating that the inclusion of L-Trp reduced fear and stress in breeder pullets (House et al., 2021). In-ovo injection of serotonin activates serotonin receptors and increases catecholamine neurotransmitters in chickens, effectively reducing fear and stress responses and promoting stable behaviour (Huang et al., 2021). In a paired house study in commercial pullet breeder farm with 9000 broiler breeder pullets, Trp was fed at about 1.5 lbs to one house with 9000 breeder pullets and 600 male pullets and compared to another house with similar male and female birds fed a normal Trp level of 0.16% Trp in the diet. A total of 160 blood samples were taken to measure the Trp during both on and off-feed days. On both on and off-feed days, the inclusion of Trp had significantly higher serotonin levels in the blood plasma of birds. The birds fed higher level of Trp in the diet had less licking and picking behaviours and reduced mortality due to aggression and piling.

Three experiments were conducted to determine if aggressive activity in developing and mature broiler breeder males is influenced by increasing dietary L-Trp. In those experiments with both developing and mature males, raised using skip-a-day feeding regime, feeding higher levels of Trp significantly decreased aggressive pecking, reduced threatening behaviour (Shea et al., 1990).

This evidently shows that increasing the Trp in the broiler breeders' diets can improve the behavioural nuisance without significantly increasing the Lys or CP content in the diet. Thus, L-Trp provides a simple way for nutritionists and breeder managers to alleviate the behavioural nuisance and improve production, fertility, and hatch without increasing the CP in the diet.



**Figure 4** Graph shows the growth trajectory of the broiler breeder male, breeder female and straight run broiler chicks

(Adopted from Aviagen parent stock and broiler guidelines, 2021 and 2022).

### *Tryptophan in broilers and Turkey*

Apart from egg production and stress response, Trp has been linked to three metabolic pathways: the first (protein), second (serotonin and melatonin), and third (kynurenine) pathways. Protein synthesis is important for optimal body weight gain and performance. Both broilers and turkeys are focused on efficient meat production. Failure to balance the essential amino acids such as Trp in low CP diets in broilers can hurt performance and profitability. In Turkey, Trp can be 4th or 5th limiting amino acid in grower and early finisher diets. However, Trp has not been considered in turkey diet formulation in the past. Limiting the Trp in Turkey can cause both performance and behavioural issues. Trp can be 7th limiting amino acid in broilers. As we reduce the CP in the diets, similar or current European feed formulation scenario, considering the optimal ratio of Trp in the diet is essential to maintain the performance of birds apart from behavioural parameters.

Two experiments were conducted on different broiler breeds, and both broiler breeds were of both sexes from 1 to 18 days of age. Six levels of Trp were fed to the birds to understand the importance of Trp. Increasing Trp improved body weight gain, feed conversion ratio (FCR), and feed intake (FI) in birds. For high-yielding birds, the Trp requirements were 0.18% for gain and 0.16% for FCR. The Trp requirement was 0.17 for body weight gain in males and females, whereas 0.17 for males and 0.16 for females for FCR (Rosa et al., 2001). As this study was done in 2001, the birds' genetic potential and development have changed in the last 14 years. As the birds are now responding even more to the amino acid densities, having an adequate amount of Trp in the diet ensures the best performance for the birds. Another study by Corzo et al. (2005) on broiler males from 42-56d of age showed that males need approximately 0.17% of dietary Trp between 42 and 56d of age for maximum performance. Opoola et al. (2017) conducted the effect of different dietary levels of Trp on the growth performance of broiler chickens in a tropical environment. The study concluded that the optimum performance was reached at 0.24% and 0.21% of dietary Trp in the starter and finisher phases, respectively (Opoola et al., 2017). A study with broiler chicken subjected under multiple stress conditions (heat stress at 30C for 10h and 23C for 14h and high stock density) inclusion of higher levels of Trp (Dig. Trp 0.38% vs. 0.19%) from 21-35d of age reduced feather corticosterone, increased trans-epithelial electric resistance, improved FCR compared to the birds with lower Trp content (Kim et al., 2024).

Tryptophan is not only important in broilers for muscles yield but also to combat environmental and physical stress. A study was conducted to evaluate the effect of Trp with broilers subjected to 2 hours of transportation stress at 42d. Birds were fed increasing Trp in the diet (0.22, 0.42, 0.62%) from 21-42d of age. Increasing Trp level in the diet decreased heat shock protein 70, corticosterone, and increased serotonin concentration (U. Bello et al., 2018). Higher Trp in the diet did not affect the breast muscle pH but decreased drip loss and increased shear force. Quantification of gut microflora showed an increase in Enterococci and Bifidobacteria populations while a reduction of E coli, Clostridia, Campylobacter and Enterobacteria populations. This study showed that Trp improved the welfare condition of the birds before and after transportation stress and shifted the balance of pathogenic/non-pathogenic bacteria towards a favourable state in the gut (U. Bello et al., 2018). The drip loss decreased and shear force increased in a dose-dependent manner by Trp supplementation, which could be due to the increase in antioxidant capacity from Trp metabolites as a result of Trp supplementation (Christen et al., 1990). A previous study indicated the same response in ducks supplemented with dietary TRP of 0.18–1.08% (Liu et al., 2015). With the kynurenine pathway, indole molecules play a significant role in the body's defence mechanism. This will help reduce the stress with disease, improve immune function and improve production. Increasing the Trp levels in the diet decreased heterophiles, monocytes, haemoglobin, and haematocrit linearly (Son et al., 2025). It has been reported that stress increases the haematocrit and WBC levels in chickens (Puvadolpirod and Thaxton, 2000). Antioxidant amino acids such as Trp can scavenge reactive oxygen species and reduce inflammation, positively influencing the immune and inflammatory response (Wu, 2009). Tryptophan metabolite, melatonin, has been known to promote the development of immune cells in chicken, and diets deficient in Trp have been shown to have reduced immune functions. Another study by Moveva et al. (2008) on stress response to chicken with supplemental Trp reported a decline in plasma heterophil: lymphocyte ratio and decreased interleukin -1Y compared to birds without Trp supplementation.

In Turkey, an increase in Leu level in the diet with the inclusion of corn and corn gluten meal replaces SBM with the hypothesis that high Leu in the diet can inhibit absorption and transport of Trp due to reduced Trp/LNAA in the diet, influencing serotonin synthesis. Unbalanced diet with high Leu and lower Trp/LNAA ratio reduced feed intake and body weight compared to birds fed a control diet. Fully balanced high Leu diets with the addition of crystalline Trp, Val, and Ile produced similar feed intake and higher body weight relative to control diets, showing the importance of Trp and balance of BCAA in turkey diets. Furthermore, plasma serotonin concentrations were lower in the uncorrected high Leu diet than in the control diet (Estanich et al., 2024, 2025).

## CONCLUSIONS

Tryptophan is an essential amino acid for poultry. Apart from being the building blocks of protein, it has several crucial metabolic and behavioural functions. It is a necessary precursor to neuroendocrine messengers such as serotonin in the gut, blood, and brain, melatonin, kynurenic acid, and quinolinic acid. Studies have found that Trp plays an essential role in regulating the insulin-like growth factor 1, corticosterone, cortisol, and secretion of heat shock protein 70. Tryptophan can help mitigate stress, feather pecking, feather licking, aggressive behaviour, cannibalism, piling, and flighty behaviour that can impact poultry welfare. Apart from stress response, Trp plays a crucial role in increasing the immune regulation against various diseases in poultry and has strong anti-inflammatory effects. In the past, Trp was supplied only from an intact protein source. Increasing Trp content in the diet through intact protein was expensive and detrimental to birds' health due to the risk of raising CP. However, with the availability of crystalline Trp, producers and nutritionists can take advantage to reap the functional benefits of Trp without adversely increasing the protein content in the diet.

