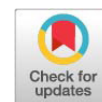


Research Article



Dietary L-Tryptophan Supplementation Improves Growth Performance, Economic Traits, and Thermoregulation in Broiler Chickens Under Heat Stress Conditions

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Abstract | Heat stress significantly impacts poultry production in tropical regions like the Philippines, where the average heat index reaches 39.84°C and is projected to increase to 4.10°C by the end of the 21st century. This environmental stress impairs broiler growth, immunity, and meat quality, leading to substantial economic losses. L-Tryptophan (Trp), an essential amino acid involved in serotonin and melatonin synthesis, may help mitigate these effects. Hence, this study investigated the effects of L-tryptophan supplementation on growth performance, body temperature, economic traits, and the correlation of Trp with body temperature, feed and water intake in broilers under heat stress. Seventy-five day-old chicks were randomly assigned to five dietary treatments in a Completely Randomized Design, with three replicates per treatment and five birds per replicate: T₁ (commercial feed, negative control), T₂ (homemade ration, [HMR] without Trp (positive control), T₃ (HMR + 0.18% Trp), T₄ (HMR + 0.20% Trp), and T₅ (HMR + 0.22% Trp). L-tryptophan supplementation significantly improved the body weight, body weight gain, average daily gain, feed intake, water intake, and feed conversion ratio (P < 0.05). Broilers in T₁ and T₂ showed elevated body temperatures and increased water intake, indicating thermal discomfort and stress (P < 0.05). Conversely, broilers supplemented with 0.20% and 0.22% L-tryptophan showed strong negative correlations in the heatmap analysis, characterized by lower body temperatures, reduced but sufficient water and feed intake. Return on investment (ROI) was highest in L-tryptophan treatments: T₅ (87.77%), T₄ (88.22%), and T₃ (80.99%), compared to T₂ (59.20%) and T₁ (60.15%). In conclusion, dietary Trp supplementation enhances broiler growth, thermoregulation, and economic viability under heat stress, making it a viable nutritional strategy for hot climates.

Keywords | Thermoregulation, Rectal temperature, Feed intake, Water intake, Feed efficiency, Economic analysis

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INTRODUCTION

The global human population is projected to increase by approximately 2 billion within the next two decades, reaching an estimated 9.7 billion by 2050 (United Nations, 2019). This demographic growth is expected to increase the demand for food, particularly for affordable and high-quality sources of animal protein (Bist *et al.*,

2024). In animal sector, poultry production has emerged as the most efficient and rapidly expanding source of animal protein worldwide, largely due to its high feed conversion efficiency, lower production costs, and shorter production cycles compared to other meat sources (OECD-FAO, 2021; Henchion *et al.*, 2021; Castro *et al.*, 2023; Vlaicu *et al.*, 2024).

In the Philippines, poultry farming is vital to food security, driven by rising per capita income, urbanization, and growing nutritional demands. In 2023, per capita poultry meat consumption reached 15.95 kg, while total domestic consumption stood at 1.934 million metric tons (MMT) (OECD-FAO, 2021). Projections estimate an increase to 2.12 MMT by 2025, a 9.6% rise over two years (IndexMundi, 2025). By 2030, per capita consumption is expected to reach 16.3 kg, with total domestic demand growing to approximately 2.287 MMT (OECD-FAO, 2021; Ordinario, 2021). This growth necessitates the optimization of production systems. However, the industry faces persistent challenges related to environmental sustainability, disease control, and animal welfare, which must be addressed to sustain productivity.

Rising temperatures and humidity levels seriously challenge poultry production, particularly in tropical countries. In the Philippines, the maritime climate frequently exposes broiler chickens to heat stress, impairing their growth, feed efficiency, and overall welfare. As of May 2025, the average heat index in Region 12 is approximately 37°C, while some regions reach up to 44°C. Climate projections suggest an increase of 0.90–4.10°C in heat index by the end of the 21st century (PAGASA, 2025). Poultry remains comfortable and can efficiently perform basic physiological functions within the temperature range of 23.9°C to 26.7°C (Bell *et al.*, 2002). Acute stress, triggered when ambient temperatures exceed 32°C, causes panting, wing spreading, and elevated respiratory and heart rates, leading to decreased feeding behavior (Wang *et al.*, 2014; Ahmad *et al.*, 2022). Heat stress raises body temperature, suppresses feed intake, and reduces weight gain and meat quality (Awad *et al.*, 2018; Sifia *et al.*, 2018). Prolonged exposure can impair thermoregulation and lead to chronic stress, further compromising productivity and overall performance (Tsiouris *et al.*, 2018; Wang *et al.*, 2014; Ahmad *et al.*, 2022).

In light of these challenges, nutritional strategies such as dietary L-tryptophan (Trp) supplementation have gained attention for their potential to mitigate heat stress effects. L-tryptophan, an essential dietary amino acid, modulates stress by boosting serotonin synthesis, which supports thermoregulation, immunity, meat quality, feed intake, feed efficiency, and lowers rectal temperature under heat stress (Young, 2013; Steenbergen *et al.*, 2016; Bai *et al.*, 2017; Kałużna-Czaplińska *et al.*, 2019; Fouad *et al.*, 2021; Zhou *et al.*, 2022). While several studies highlight the benefits of tryptophan supplementation on growth performance, El-Gogary and Azzam (2014) reported no significant effect on body weight gain or feed conversion ratio in broilers. Moreover, studies evaluating broiler chickens raised under heat stress conditions in the Philippine setting remain limited, and economic traits are often not documented.

Hence, this study investigates the effects of Trp supplementation on growth performance, economic traits, and body temperature of broilers raised under heat stress conditions. The findings aim to inform the development of feeding technology for heat-stressed broilers and support knowledge transfer to enhance poultry production in tropical climates.

MATERIALS AND METHODS

PROCUREMENT OF EXPERIMENTAL BIRDS

The study was carried out following the standard rearing of farm animals as stipulated in the good animal husbandry practices of the Philippines concerning animal farming, health, and welfare (PNS/BAFPS, 2008). One hundred (100) day-old Cobb 500 broiler chicks were procured from a reliable source and housed in the Animal Science experimental area of the College of Agriculture, Sultan Kudarat State University, Lutayan Campus.

HOUSING AND EXPERIMENTAL MANAGEMENT

One week before the chicks' arrival, the entire facility including the brooder pen, an elevated colony-type structure measuring 1 meter in width by 2 meters in height was thoroughly cleaned and disinfected with a commercially available product to minimize the risk of disease. Upon arrival, the day-old chicks were placed in the brooder pen, where newspapers were used as bedding and regularly changed to prevent manure buildup. Two 50-watt electric bulbs provided artificial heat until the chicks could regulate their own body temperature. During this 10-day brooding period, broiler chicks were fed *ad libitum* using the commercial chick booster mash.

On day 11, the chicks were transferred to grower cages, also elevated colony-type pens made from locally available materials, with bamboo slat walls and screen mesh flooring meeting the standard floor space requirement of 1 square foot per bird. From this point, the broiler experimental starter and finisher rations were followed. The experimental birds were fed starter mash from day 11–24 and then gradually transitioned to finisher mash from day 25–38, following the provided experimental rations throughout the feeding trial. Fresh, clean drinking water was provided throughout the feeding experiment, and all birds were treated equally with regard to other environmental requirements (Minanga and Ampode, 2021). Routine sanitation practices, including daily dung removal, were implemented to maintain cleanliness and control flies and foul odors.

To simulate heat stress conditions, the experimental house and cages were covered with tarpaulin to limit air circulation, and additional artificial lighting was used to

raise the ambient temperature. The housing environment was monitored three times daily at 6:00 AM, 12:00 NN, and 6:00 PM using a digital hygrometer (Curi *et al.*, 2017). The average ambient temperature during the study was 41.71°C, with a relative humidity of 32%. Temperature–humidity index (THI) values from day 11 to day 31 were used to assess the impact of heat stress. The calculated Temperature-Humidity Index (THI) was 88.72, indicating severe heat stress in broilers. THI was computed using the formula by Boonkum *et al.* (2025), adapted from NOAA (1976): $THI = (1.8 \times T + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)$.

HEALTH MANAGEMENT

A standard dose of electrolytes and multivitamins was administered through the drinking water seven days before the start of the experiment to strengthen the birds' immune system against management-related stress. The experimental pens were disinfected using a standard commercial disinfectant solution, and the process was completed seven days prior to the start of the experiment to allow for a proper downtime period. Appropriate biosecurity measures were observed, including the installation of fences to prevent unauthorized individuals or stray dogs from interfering with the experiment.

PREPARATION OF HOMEMADE RATION

A feed formulation was developed based on the proximate analysis of the collected feedstuff samples. Nutrient requirements of broiler chickens were aligned with the guidelines provided in the Philippine Recommends for Livestock Feed Formulation (PCAARRD, 2000) to ensure a balanced and adequate nutrient supply throughout the study. Two dietary phases were formulated to meet the physiological needs of the birds: A starter ration containing 20% crude protein (CP) and a finisher ration with 18% CP. The homemade ration (HMR) consisted of ground yellow corn, rice bran D1, soybean meal, fish meal, copra meal, dicalcium phosphate, limestone, lysine HCl, DL-methionine, L-tryptophan, vitamin premix, salt, and vegetable oil. All experimental birds received isocaloric and isonitrogenous diets, ensuring that each treatment group was supplied with equal energy and protein levels. The calculated nutrient composition of the formulated diets is presented in Table 1.

EXPERIMENTAL DESIGN AND TREATMENT

A total of seventy-five day-old broiler chicks, regardless of sex, were used in the study. The chicks were randomly assigned to five dietary treatment groups, with each treatment replicated three times and five birds per replicate. The experiment followed a Completely Randomized Design. The dietary treatments were as follows: T₁: Commercial ration; T₂: homemade ration (HMR) without

Trp; T₃: HMR + 0.18% Trp; T₄: HMR + 0.20% Trp; and T₅: HMR + 0.22% Trp. The negative control (T₁) used a commercial feed that typically includes essential amino acids and antibiotics, representing the standard industry diet. The positive control (T₂) consisted of a homemade ration without Trp and antibiotics, serving as a baseline to assess the specific effects of Trp supplementation against a simplified diet lacking these additives.

Table 1: Calculated nutrient composition of homemade starter and finisher rations.

Calculated analysis	Starter 11–24 days	Finisher 25–38 days
Crude Protein, %	20.00	18.00
Metabolizable Energy, Kcal	2848.91	2883.31
Avail Phosphorus, %	0.49	0.49
Calcium, %	0.87	0.99
Lysine, %	1.35	1.08
Methionine, %	0.45	0.45
Met+Cys, %	0.64	0.67
Vitamin Premix ¹ , %	0.90	0.90

¹Vitamin Premix (fine quality/texture; 500 g contains) dicalcium phosphate 97%, Vitamin 150,000 units, Vitamin D3 30,000 units, Vit. E 500 I.U., Potassium Iodine 100mg, Carrier q.s. ad 500mg, Manganese Sulfate 3,500mg, Ferrous Sulfate 1,500mg, Copper Sulfate 1,500mg, Cobaltous Sulfate 30mg, Zinc Sulfate 200mg.

DETERMINATION OF WATER INTAKE, BODY TEMPERATURE, AND NET INCOME

Clean drinking water was provided *ad libitum* throughout the experimental period. Water intake (ml) was measured daily by recording the total volume of water offered and subtracting the remaining volume to determine the actual consumption per group. Body temperature was monitored three times daily at 6:00 AM, 12:00 PM, and 6:00 PM, by recording the cloacal temperature of each bird using a digital thermometer. These readings were averaged and presented on a per-day basis. Economic analysis was also calculated to evaluate profitability. Gross income Gross income referred to the total revenue from broiler sales. Net income or Return Above Feed and Chick Cost (RAFCC), was calculated by deducting the cost of feed and day-old chicks from the gross income. Return on Investment (ROI) was computed as: $ROI = (\text{Net Profit} / \text{Total Investment}) \times 100$ (Zacaria and Ampode, 2021).

STATISTICAL ANALYSIS

Data gathered were subjected to one-way analysis of variance (ANOVA), and treatment means were compared using Tukey's Honest Significant Difference (HSD) Test using Statistical Tool for Agricultural Research software. A p-value of less than 0.05 was considered significant.

The heatmap correlation of the housing temperature with the feed intake, water intake, and body temperature per day was analyzed using the MetaboAnalyst 5.0 software. The R^2 values falling within the range of 0 to 30 indicated weak association; those in the 0.31 to 60 range suggested moderate association, while values from 0.61 to 1.00 signified strong association.

RESULTS AND DISCUSSION

BODY WEIGHT

The effects of the dietary supplementation of Trp on the growth performance of broiler chickens raised under heat stress conditions are presented in Table 2. The initial weight of the experimental birds showed no significant difference among treatment means. The cumulative final

body weight results across the three phases showed a clear advantage for broilers raised under heat stress and fed Trp-supplemented diets ($P < 0.05$), although the values were statistically comparable to those of the T_1 group fed a commercial ration (negative control). On day 24, no significant differences were observed in body weight across all treatments, indicating that dietary changes did not immediately influence early growth. However, by day 38, significant differences were observed ($P < 0.05$). T_5 (0.22% Trp) had the highest body weight, followed by T_1 , T_4 , and T_3 , while T_2 (0% Trp) recorded the lowest body weight. The difference between T_2 and the Trp supplemented groups underscores the role of Trp in mitigating the negative effects of heat stress, likely through improved gut integrity and reduced corticosterone levels.

Table 2: Effect of dietary L-tryptophan supplementation on the growth performance of broiler chickens raised under heat stress conditions.

Parameters	Treatments					P-value	CV
	T ₁	T ₂	T ₃	T ₄	T ₅		
Body weight, g							
Initial Weight	181.27	182.00	181.73	181.63	182.13	0.600 ^{ns}	0.38
11-24, days	675.00	647.53	679.40	714.67	720.27	0.270 ^{ns}	3.29
25-38, days	1319.60 ^a	1173.80 ^b	1295.53 ^a	1301.73 ^a	1348.87 ^a	0.001 [*]	1.58
11-38, days	1319.60 ^a	1173.80 ^b	1295.53 ^a	1301.73 ^a	1348.87 ^a	0.001 [*]	1.58
Body weight gain, g							
11-24, days	493.73	465.53	497.67	533.07	538.13	0.276 ^{ns}	3.86
25-38, days	644.60	526.27	616.13	587.07	628.60	0.187 ^{ns}	9.76
11-38, days	1138.33 ^a	991.80 ^b	1113.80 ^a	1120.13 ^a	1166.73 ^a	0.001 ^{**}	1.82
Average daily gain, g							
11-24, days	35.27	33.25	35.55	38.08	38.44	0.275 ^{ns}	3.86
25-38, days	46.04	37.59	44.01	41.93	44.90	0.187 ^{ns}	9.74
11-38, days	81.31 ^a	70.84 ^b	79.56 ^a	80.01 ^a	83.34 ^a	0.001 ^{**}	1.82
Feed intake, g							
11-24, days	714.93 ^b	719.27 ^b	726.80 ^b	731.93 ^b	1030.93 ^a	0.001 ^{**}	3.69
25-38, days	1080.33 ^a	1045.27 ^a	907.40 ^b	916.93 ^b	890.00 ^b	0.001 ^{**}	4.60
11-38, days	1795.27 ^a	1777.53 ^a	1634.20 ^b	1648.87 ^b	1612.67 ^b	0.001 [*]	1.49
Water intake, ml							
11-24, days	7104.53 ^a	7164.13 ^a	7150.87 ^a	6759.93 ^b	5683.94 ^c	0.001 ^{**}	1.12
25-38, days	9877.80 ^{ab}	10116.87 ^a	9793.87 ^{ab}	9429.27 ^b	7701.89 ^c	0.001 ^{**}	2.66
11-38, days	16982.40 ^a	17282.40 ^a	16944.81 ^a	16189.31 ^b	13385.83 ^c	0.001 ^{**}	1.59
Feed conversion ratio							
11-24, days	1.45 ^a	1.55 ^a	1.46 ^a	1.38 ^a	1.84 ^b	0.001 ^{**}	4.11
25-38, days	1.68 ^a	1.99 ^b	1.48 ^a	1.57 ^a	1.42 ^a	0.001 ^{**}	21.25
11-38, days	1.58 ^b	1.79 ^c	1.47 ^a	1.47 ^a	1.38 ^a	0.001 ^{**}	2.05

CV: Coefficient of variation; ^{ab}Letter superscript within row is significantly different at $p < 0.05$; ^{ns}not significant ($p > 0.05$); * means with different superscripts within row is significantly different ($P < 0.05$); ** means with different superscripts within row is significantly different ($P < 0.01$); T_1 : Commercial ration (negative control); T_2 : HMR without Trp and SA (positive control); T_3 : HMR + 0.18% Trp; T_4 : HMR + 0.20% Trp; T_5 : HMR + 0.22% Trp

The results of this study contradict those of El-Gogary and Azzam (2014), who found that increasing dietary tryptophan had no significant effect on broilers' body weight, weight gain, or feed conversion ratio. However, the recent findings were supported by Ouyang *et al.* (2023), who confirmed that Trp modulates serotonin production, which regulates stress responses and improves growth under environmental stressors. The authors further explained that dietary 0.18% Trp supplementation protects against acute heat stress-induced oxidative stress and mitochondrial dysfunction in broilers by regulating antioxidant states and increasing mitochondrial function-related gene expression. Further, Fouad *et al.* (2021), reported that Trp supplementation in poultry diets can optimize productivity, immune system, antioxidant status, and meat and egg quality, potentially benefiting meat and egg producers. This suggests Trp improved nutrient metabolism and enhances the birds' resilience to heat stress, promoting better growth.

BODY WEIGHT GAIN

The cumulative mean body weight gain and daily average gains of the experimental birds raised under heat stress conditions supplemented with dietary Trp showed a pattern similar to that of body weight, with significant differences observed during the overall period ($P < 0.05$). No significant differences were observed among treatments from days 11–24 ($P > 0.05$), although T_4 and T_5 showed numerically higher weight gains. Similarly, from days 25–38, while differences remained statistically insignificant, T_5 showed the highest gain and T_2 the lowest. Overall, from days 11–38, T_5 recorded the highest cumulative gain, significantly better than T_2 , while T_3 and T_4 also showed substantial improvement but were statistically comparable with T_1 .

Average daily gain (ADG) showed statistically significant differences ($P < 0.05$) in the overall period (11–38 days), with T_5 achieving the highest ADG and T_2 the lowest. In the earlier phase (11–24 days), differences were not significant ($P > 0.05$), though birds in T_5 and T_4 numerically led the group. The later phase (25–38 days) again favored T_5 and the other Trp supplemented groups, but their performance was statistically comparable to that of the T_1 group fed a commercial ration (negative control). The consistent improvement in ADG in Trp-supplemented groups suggests a cumulative effect of the amino acid in reducing oxidative stress, regulating appetite, and improving energy utilization. Ouyang *et al.* (2023) found that Trp can mitigate the adverse effects of chronic heat stress in broilers by enhancing antioxidant enzyme activities, reducing inflammatory responses, and improving mitochondrial function, thereby potentially stabilizing feed intake and promoting growth. These findings are consistent with Kim *et al.* (2024), who reported enhanced protein synthesis and muscle development in heat-stressed broilers supplemented with tryptophan. Further,

Mund *et al.* (2019) and Yue *et al.* (2017) reported that supplementation of dietary Trp improved the body weight gain, feed conversion, immune function, and antioxidant capacity in broilers, particularly under oxidative and chronic stress conditions. These findings confirm the importance of Trp in sustaining consistent growth across all production phases under thermal stress. This suggests that Trp buffers against heat stress and actively supports growth through physiological mechanisms associated with amino acid metabolism.

FEED INTAKE

Feed intake results showed statistically significant differences ($P < 0.05$) across all time periods days 11–24, 25–38, and the overall 11–38 days, highlighting how dietary Trp influenced consumption patterns under heat stress. From days 11–24, feed intake was significantly higher in T_5 compared to all other treatments. This suggests that the highest level of Trp (0.22%) initially stimulated appetite, likely due to its role in serotonin synthesis, which regulates feeding behavior and mood (Steenbergen *et al.*, 2016; Young, 2013). L-tryptophan supplementation may have enhanced hypothalamic serotonin levels, promoting greater feed consumption in a heat-stressed environment. According to Haleem (2017), serotonin precursors like Trp can alleviate stress-induced anorexia by modulating neurotransmitters associated with satiety and anxiety.

In the later stage (days 25–38), an interesting shift was observed where birds in the Trp groups (T_3 – T_5) had significantly lower feed intake than T_1 and T_2 . Broilers in T_5 , despite its initial high intake, consumed only 890.00g during this phase, less than T_1 (1080.33 g) and T_2 (1045.27 g). This pattern implies improved nutrient utilization and metabolic efficiency in birds fed with Trp. They required less feed to achieve better growth performance, as supported by their superior weight gain and FCR. Maharjan *et al.* (2020) highlighted that amino acid supplementation improves gut health and enzyme efficiency, reducing the energy needed for maintenance and thus lowering feed demand in stressful environments.

Overall (11–38 days), T_1 and T_2 had the highest cumulative feed intake, while the Trp-supplemented treatments consumed less. Despite the lower intake, T_5 achieved the highest body weight and gain, demonstrating a significant improvement in feed efficiency. This implies that dietary L-tryptophan not only reduces stress-related anorexia but also enhances feed utilization, reducing the need for excess consumption to achieve target growth.

WATER INTAKE

Water is a critical nutrient for maintaining thermoregulation, digestion, nutrient transport, and waste elimination in broilers (Jafari *et al.*, 2016), understanding its interaction

with dietary strategies is essential for improving poultry resilience to heat stress. Results showed statistically significant differences ($P < 0.05$) across all periods days 11–24, 25–38, and the overall 11–38 days indicating that dietary Trp influenced water consumption patterns in broilers under heat stress. Intake was significantly higher in T_2 , but statistically comparable to T_1 and T_3 , while birds in T_4 and T_5 had lower water intake. Despite this reduction, water consumption in T_4 and T_5 remained sufficient to support optimal growth, suggesting that Trp supplementation may help mitigate dehydration by moderating stress-related water loss.

Heat-stressed birds often display behavioral changes such as panting and restlessness, which lead to increased water intake. In this study, the average daily water consumption (ml) per bird was 606.51, 617.23, 605.17, 578.19, and 478.06 for treatments T_1 , T_2 , T_3 , T_4 , and T_5 , respectively. These values are numerically higher than those reported in previous studies, such as Sinkalu *et al.* (2020), who recorded an average intake of 391.86 ml per bird over six weeks, and Williams *et al.* (2013), who reported 313.39 ml per day during a 38-day rearing period. However, these earlier studies were conducted under normal environmental conditions. The elevated water consumption in the present study is likely attributed to birds being exposed to heat stress, which increases the physiological demand for evaporative cooling through panting. Notably, birds fed with Trp-supplemented diets showed lower water intake compared to non-supplemented groups, suggesting that tryptophan may help alleviate heat-induced stress responses. The inclusion of Trp in the diet may reduce the severity of dehydration and limit excessive compensatory drinking, thereby supporting better hydration balance under heat stress conditions.

FEED CONVERSION RATIO

The feed-conversion ratio (FCR) is vital in measuring broiler performance. The feed conversion ratio was significantly improved ($P < 0.05$) in birds supplemented with Trp, particularly in T_3 to T_5 , across all periods. In the overall period (11–38 days), T_5 had the best FCR, followed closely by T_3 and T_4 , while T_2 recorded the poorest efficiency. FCR is a rate measuring the efficiency with which the bodies of livestock convert animal feed into the desired output, which means that the lower the value, the more efficient the birds are in converting feed to live weight.

The significant differences highlight the improved feed utilization efficiency in birds receiving dietary Trp, even under high environmental temperatures. T_5 's high feed intake during early growth (days 11–24) balanced out with efficient nutrient use in later phases, resulting in optimal FCR. Conversely, El-Gogary and Azzam (2014)

reported that increasing dietary tryptophan doesn't always affect body weight, weight gain, or feed conversion ratio. However, the recent findings were supported by Mund *et al.* (2020), who emphasized that dietary Trp supplementation improved nutrient digestibility and reduced maintenance energy needs, leading to better FCR. This indicates that Trp plays a metabolic role in maximizing the return on feed investment during critical production phases.

However, it is important to note that excessive supplementation of amino acids like Trp can lead to metabolic inefficiencies, as surplus amino acids cannot be stored and are deaminated, with nitrogen excreted as uric acid. In poultry litter, uric acid converts to urea, which hydrolyzes to NH_3 , some of which protonates NH_4^+ (Liang *et al.* 2014). This microbial breakdown increases ammonia emissions, potentially harming air quality and bird welfare (Nahm, 2005; Wang *et al.*, 2014). Therefore, while Trp improved feed efficiency and thermoregulation, over supplementation should be avoided.

Table 3: Effect of L-tryptophan supplementation on the body temperature of broiler chickens raised under heat stress conditions.

Parameters	Treatments					P value	CV
	T_1	T_2	T_3	T_4	T_5		
Body temperature	42.09 ^b	43.81 ^c	41.62 ^{ab}	40.91 ^{ab}	40.18 ^a	0.004**	2.07

CV: Coefficient of variation; ^{abc}Letter superscript within row is significantly different at $p < 0.05$; **means with different superscripts within row is significantly different ($P < 0.01$); T_1 : Commercial ration (negative control); T_2 : HMR without Trp and SA (positive control); T_3 : HMR + 0.18% Trp; T_4 : HMR + 0.20% Trp; T_5 : HMR + 0.22% Trp

BODY TEMPERATURE

Broiler chickens raised under heat stress and fed diets supplemented with L-tryptophan (T_3 , T_4 , T_5) exhibited significantly lower rectal body temperatures compared to those receiving either a commercial ration (T_1) or HMR without tryptophan (T_2 , Table 3). Birds in the Trp-supplemented groups recorded rectal temperatures of 41.62°C (T_3 : 0.18% Trp), 40.91°C (T_4 : 0.20% Trp), and 40.18°C (T_5 : 0.22% Trp). In contrast, higher body temperatures were observed in T_1 (42.09°C) and especially in T_2 (43.81°C). The statistical analysis shows that T_2 was significantly different from all other groups, while T_5 differed significantly from both T_1 and T_2 . Birds in T_3 and T_4 were statistically comparable to T_1 and T_5 , indicating a graded response to increasing tryptophan levels. These findings demonstrate the thermoregulatory benefits of dietary tryptophan under heat stress conditions. Tryptophan likely contributes to this effect by acting as a biochemical precursor to serotonin (5-hydroxytryptamine), a neurotransmitter involved in regulating body temperature.

Li *et al.* (2022) observed that intravenous administration of tryptophan reduced rectal temperature in heat-stressed steers, attributed to elevated central serotonin levels.

In poultry, heat stress impairs the normal thermoregulatory balance, leading to elevated core body temperature and compromised physiological functions. Increased serotonin synthesis via dietary tryptophan may modulate hypothalamic activity, enhance heat dissipation, and reduce metabolic heat production through mechanisms such as lower physical activity or altered neuroendocrine responses. Moreover, serotonin modulates the hypothalamic-pituitary-adrenal (HPA) axis, a central player in the stress response (Smith and Vale, 2006). Through this neurochemical pathway, L-tryptophan likely supports a more stable thermoregulatory state, improving resilience to thermal stress.

CORRELATION OF L-TRYPTOPHAN SUPPLEMENTATION WITH PERFORMANCE PARAMETERS

The heatmap illustrates the effects of various dietary treatments on broiler chickens subjected to heat stress,

comparing three key physiological parameters: Body temperature, water intake, and feed intake (Figure 1). The intensity and color gradient reflect standardized values, with red shades denoting positive correlations (higher values) and blue shades indicating negative correlations (lower values).

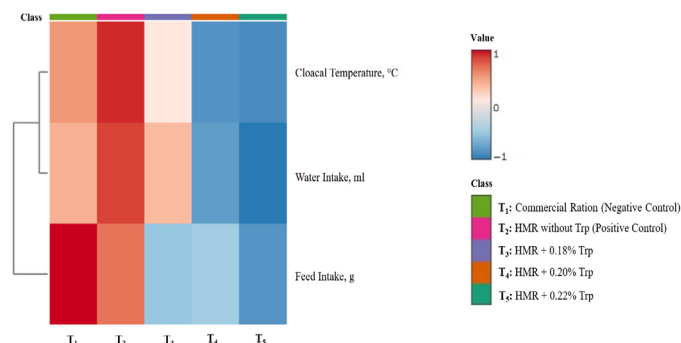


Figure 1: Correlation of L-Tryptophan supplementation with performance parameters in broiler chickens under heat stress conditions; red shades represent positive (higher) correlations, and blue shades indicate negative (lower) correlations.

Table 4: Effect of L-tryptophan supplementation on the return above feed and chick cost of broiler chickens raised under heat stress conditions.

Parameters	Treatments				
	T ₁	T ₂	T ₃	T ₄	T ₅
Final live weight, kg	1319.60	1173.80	1295.53	1301.73	1348.87
Price/kg live weight, PhP	160.00	160.00	160.00	160.00	160.00
Gross return/head, PhP	211.14	187.81	208.28	208.28	215.82
Cost of DOC/head, PhP	38.00	38.00	38.00	38.00	38.00
Feed consumption, g/head					
a. CBM ¹	45.00	45.00	45.00	45.00	45.00
b. Starter	715.00	719.00	732.00	732.00	723.00
c. Finisher	1080.00	1045.00	917.00	917.00	890.00
Price/kg of Feed, PhP					
a. CBM	49.00	49.00	49.00	49.00	49.00
b. Starter	41.50	33.62	33.62	33.62	33.61
c. Finisher	39.00	33.01	33.01	33.01	33.01
Total feed cost, PhP					
a. CBM	22.05	22.05	22.05	22.05	22.05
b. Starter	29.67	24.17	24.44	24.61	24.30
c. Finisher	42.12	34.50	29.94	30.27	29.38
Total cost, PhP	131.84	118.72	114.43	114.93	113.73
RAFCC ¹	79.30	69.09	92.86	93.35	102.09
ROI, % ³	60.15	59.20	80.99	81.22	87.77

¹CBM: Chick Booster Mash; ²RAFCC: Return Above Feed and Chick Cost was computed by adding all the total cost (chick cost, feed cost); ³ROI: Return on Investment; PhP: Philippine Peso; T₁: Commercial ration (negative control); T₂: HMR without Trp and SA (positive control); T₃: HMR + 0.18% Trp; T₄: HMR + 0.20% Trp; T₅: HMR + 0.22% Trp

The heatmap reveals clear correlation patterns in the daily physiological responses of broilers exposed to uniform heat stress conditions. A positive correlation was observed between body temperature and water intake in the negative control (T_1) and HMR without Trp (T_2) groups, indicating that elevated body temperatures triggered increased water consumption an expected thermoregulatory response to heat stress. In contrast, Trp supplementation at 0.20% and 0.22% (T_4 and T_5) was negatively correlated with both body temperature and water intake. This suggests that higher Trp inclusion effectively reduced thermal load and alleviated stress-related physiological responses. This heatmap is based on daily measurements of body temperature, water intake, and feed intake across all treatment groups.

While feed intake decreased progressively in the HMR with Trp supplementation compared to the control, a negative correlation between Trp levels and feed intake did not translate to impaired growth performance. On the contrary, broilers supplemented with Trp exhibited improved weight gain, indicating that their reduced feed intake was still sufficient to meet their energy and nutrient requirements for optimal growth. This highlights the potential of Trp to enhance feed efficiency under heat stress conditions. The clustering and color distribution in the heatmap further reinforce the beneficial role of Trp in mitigating heat-induced physiological strain. These findings align with [Lara and Rostagno \(2013\)](#), who reported that elevated ambient temperatures generally increase water consumption and elevate body temperature in broilers as part of their thermoregulatory adaptation.

RETURN ABOVE FEED AND CHICK COST

The Return Above Feed and Chick Cost (RAFCC) refers to the amount gained using the treatment of the study ([Table 4](#)). Among the five dietary treatments, T_5 HMR + 0.22% Trp had the highest return of 102.09 PhP, T_4 followed this with 93.35 PhP ; T_3 with 92.86 PhP; T_1 with 79.30 PhP and T_2 with 69.09 PhP. Similarly, return on investment (ROI) was highest in Trp treatments: T_5 (87.77%), T_4 (88.22%), and T_3 (80.99%), compared to T_2 (59.20%) and T_1 (60.15%). The results demonstrate that dietary treatments containing Trp, specifically T_5 with .22% and T_4 with .20%, led to higher returns compared to other treatments. This suggests that incorporating Trp into broiler diets can improve profitability, making it a beneficial addition for poultry farmers. This could lead to even greater improvements in profitability and broiler performance.

CONCLUSION

The study revealed that dietary supplementation with Trp, particularly at 0.20–0.22%, significantly enhanced

growth performance, feed efficiency, and economic returns in broiler chickens under heat stress, while also reducing body temperature and improving thermoregulation. These findings indicate that Trp enhances stress resilience, nutrient utilization, and overall profitability, supporting its recommended inclusion in broiler diets under heat stress conditions. Further research is recommended under commercial production settings with controlled environments, such as chambered poultry houses, to better monitor environmental factors. Additionally, feeding trials in layer chickens should be conducted to evaluate the effects of Trp on laying performance, egg quality, and behavior, to gain deeper insights into its role in stress mitigation and animal welfare.

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NOVELTY STATEMENT

This investigation offers novel insights into the physiological and economic benefits of L-tryptophan supplementation in broilers raised under heat stress condition. By linking growth performance, thermoregulation, and profitability, the study fills a critical gap in context-specific nutritional strategies for sustainable poultry production in hot climates.

AUTHORS CONTRIBUTION

Both authors contributed equally to the conceptualization, data collection and interpretation, statistical analysis, and manuscript writing, and are recognized as co-first authors of this manuscript.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

The authors declare that AI-assisted tools (ChatGPT and Grammarly) were used solely to improve English language quality and grammar. All outputs were reviewed by the authors. No AI tools were used for data analysis or interpretation.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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