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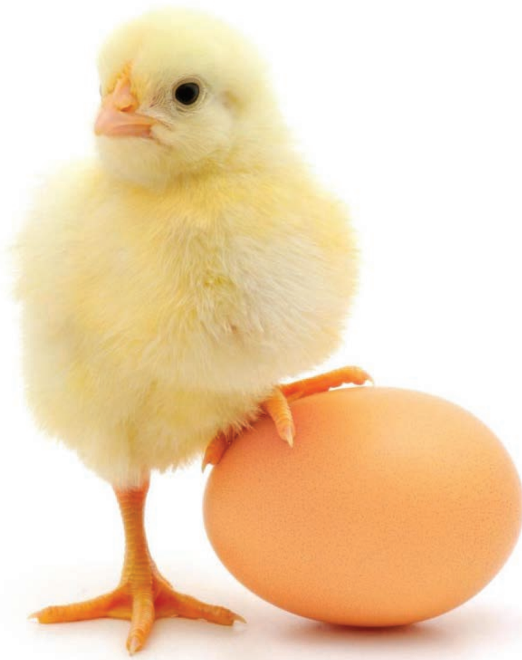

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# Research Article
# Effect of Dietary Amino Acids Levels on Growth Performance and Water/Feed Ratio in Cobb Broiler Chickens under Heat Stress

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## Abstract

**Objective:** This experiment aimed to investigate the effect of different dietary amino acids levels on growth performance and mortality of Cobb broilers under heat stress. **Materials and Methods:** A total of 800 one-day-old Cobb 500 broilers were randomly assigned to 4 treatment groups [Non heat stress control group (NHC), Heat stress control group (HC), Heat stress low amino acid (HLA) and Heat stress high amino acid treatment group (HHA)], each with 8 replicates and 25 broilers per replicate. The house temperature in NHC group was applied in accordance with routine feeding management, while others experienced cyclic heat stress. **Results:** Results indicated that, during 0-7 day, there were no significant differences in growth performance, mortality and water consumption among the four groups ($p>0.05$). From day 8-28, growth performance was significantly lower ($p<0.05$), while mortality was significantly higher in the three heat stress groups (HC, HLA and HHA group) compared with NHC group ($p>0.05$). Compared to the HC groups, in the HLA group feed intake (FI) during days 8-28, average body weight at day 28 and European Efficiency Index (EPI) was significantly higher, with significantly lower mortality. In the HHA group, FI during days 8-28, average body weight at day 28 and EPI were significantly lower, with significantly higher mortality compared to other groups. Additionally, water consumption and the total water/feed ratio of broilers in the three heat stress treatment groups were significantly higher than the NHC group during days 1-28 ($p<0.05$). **Conclusion:** Heat stress significantly influenced the growth performance and increased mortality of Cobb broilers from 8-28 days. Higher levels of dietary amino acids can exacerbate the effects of heat stress, while lower levels of dietary amino acid improved growth performance, reduced mortality and ultimately enhanced economic efficiency.

Key words: Cobb broiler, digestible amino acid, growth performance, heat stress, mortality, water/feed ratio

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Indonesia is a Southeast Asian country with a typical tropical monsoon and tropical rainforest climate, with an annual average daily temperature of around 30°C and humidity exceeding 70%. The broiler chicken breeding industry in Indonesia has a relatively low level of intensification and the predominant model involves collaboration between companies and individual farmers. Breeding methods commonly include ground-level, open chicken houses, especially among small-scale or home-based breeders due to the relatively lower initial costs[1]. The persistent high temperatures and humidity, coupled with outdated breeding equipment, contribute to the frequent occurrence of heat stress among broiler chickens. Heat stress leads to decreased growth performance and an increased mortality rate in broiler chickens, resulting in substantial economic losses for both farmers and the Indonesian broiler breeding industry.

Chickens possess a highly developed heat regulation mechanism. Broilers maintain their normal body temperature at 10-32°C and 21-26°C is the comfortable ambient temperature range for survival and broilers can achieve peak growth performance. While the temperature range of 26-32°C can sustain normal physiological functions, it adversely affects production performance. When the ambient temperature exceeds the upper limit of the broilers' isothermal range (32°C), the body initiates various non-specific responses, a condition referred to as heat stress[2]. Earlier studies indicated that adjusting dietary nutrient levels[3], using additives[4], administering anti-heat stress drugs[5], or altering feeding methods[6] can mitigate the impact of heat stress on broilers to some extent. Nevertheless, nutritional regulation for enhancing the growth performance of animals under heat stress is considered the most effective approach, representing a focal point in the field of animal nutrition.

It is controversial to adjust the energy or protein level of broiler diets to achieve optimal growth performance under heat stress. The early findings suggested that increasing the concentration of dietary energy and protein under high-temperature conditions could enhance feed intake and final weight of broilers[3,7].

However, some studies suggested that feeding high protein diets in high-temperature environments can increase heat production during protein metabolism, which not only fails to alleviate heat stress but also results in reduced poultry growth performance and increased mortality rate[8,9]. Additionally, studies indicated that feeding diets with the same energy level but varying lysine (Lys) levels under heat stress resulted in notable differences in feed intake and final weight. However, by assessing lysine levels only, we cannot extrapolate the alterations in broiler growth performance under heat stress when the overall amino acid levels of the diet change. In this experiment, digestible amino acids in broiler chicken diets were examined. Three dietary treatments were designed with varying digestible amino acid levels and supplementing exogenous amino acids to achieve dietary amino acid balance. The objective was to explore the effects of feeding diets with different digestible amino acid levels on growth performance, mortality rate and Water/Feed ratio of broilers under heat stress. This study provides foundation for the formulation of broiler diets in high-temperature and high-humidity environment in Indonesia.

## MATERIALS AND METHODS

**Experimental design and birds management:** A total of 800 one-day-old COBB 500 broiler chickens with similar health conditions (average weight 42.7±0.34 g) were randomly allocated into four groups: Non heat stress control group (NHC), Heat stress control group (HC), Heat stress low amino acid (AA) treatment group (HLA) and Heat stress high amino acid treatment group (HHA). Each treatment had eight replicates, with 25 chickens per replicate, half male and half female. The birds were housed in floor pens (20 m×10 m, 200 $m^2$) as groups in random manner, each pen was enclosed by thick wooden boards to create a confined space, equipped with heating and cooling systems, feeders, separate water supply systems and meters to monitor daily water consumption. The environment was maintained with 24 hrs light, proper air ventilation and under standard management conditions. The experimental treatments initially received identical diets for days 0 to 7. Subsequently, the NHC and HC groups were fed a basic diet with Digestible Lysine (DLys) at 1.12%, the HLA group received a low amino acid diet with DLys at 1.02% and the HHA group received a high amino acid diet with DLys at 1.22% from days 8-28. Broilers in NHC group followed a standard temperature program followed the breeder's recommendations (Cobb, 2021) throughout the study. To mimic the high temperature and high humidity conditions in Indonesia, the Heat stress treatment groups (HC, HLA and HHA) followed the cyclic heat stress model proposed by Liu *et al.*[10]. Daily, the temperature increased from 7:00 am, reaching approximately 35 by 9:00 am and remained at 35±2°C for 8 house until 17:00. Subsequently. it gradually decreased, reaching 30°C from 19:00 until 7:00 the next day. The temperature was maintained at 30±2°C for 12 hrs and the relative humidity of the chicken house was controlled at

70-80%. Electric heat pipes provided heating for the experimental treatments and a temperature controller was employed for temperature regulation. A humidifier and water spray controlled the relative humidity of the chicken house. Temperature and humidity were recorded throughout the day with maximum and minimum temperature meters, along with dry and wet bulb thermometers. Temperature and relative humidity for each group were recorded three times daily. The experiment was conducted at the experimental farm of PT. New Hope Indonesia Cirebon (Cirebon, West Java, Indonesia) and spanned a duration of 28 days. The experimental design and treatments are shown in Table 1.

**Dietary treatment:** A two-phased experimental diet was formulated: 1-7 days for the first phase and 8-22 days for the second phase. The basal diet was formulated according to the Nutritional Requirements of COBB (COBB, 2022) and the New Hope Indonesia Nutritional recommendation standard for broiler chickens. It contained DLys at 1.22% for 0-7 days and 1.12% for 8-28 days. For 8-28 days, DLys was reduced by 9-1.02% in the low amino acid group, while the DLys increased by 9-1.22% in high amino acid group. Simultaneously, in order to achieve a balanced digestible amino acid profile in the diet, levels of other essential amino acids, including Methionine, Cystine, Tryptophan and Threonine, were adjusted based on the digestible amino acid balance standard for COBB broilers outlined in Table 2. Aside from variations in amino acid and crude protein levels, each treatment group maintained uniform nutritional concentration and used identical raw material. The details of dietary ingredients and nutrient composition of experimental diets are presented in Table 3.

### Traits measured and methods

**Growth performance:** In order to determine the initial weight, replicates were weighed prior to the experiment and the final weight was recorded on the 7th and 28th days of the experiment. Feed consumption for each replicate was calculated 7 days before the experiment and the whole period and the feed consumption for dead chickens and eliminated chickens was corrected. The average body weight of broilers at 7 days and 28 days and the average daily gain (ADG), average daily feed intake (ADFI) and feed conversion rate (FCR) of broilers during 1-7 days, 8-28 days and 1-28 days were computed using the following formulas:

$$\text{Average daily gain (ADG, g/day)} = \frac{\text{Final weight-Initial weight}}{\text{Trail days}}$$

$$\text{Average daily feed intake (ADFI, g/day)} = \frac{\text{Total feed consumption}}{\text{Trail days}}$$

$$\text{Feed conversion rate (FCR)} = \frac{\text{Total feed consumption}}{\text{Final weight-Initial weight}}$$

**Mortality rate and European efficiency index (EPI):** The number of dead and culled chickens were recorded every day. The total count of dead and culled chickens for the first 7 days and the entire period was tallied and mortality rates for 1-7 days and 1-28 days were computed. The mortality rate and European efficiency index (EPI) for each group were calculated using the following formula:

$$\text{Mortality rate (\%)} = \frac{\text{Number of dead and culled chickens}}{\text{Initial total number of chickens}} \times 100\%$$

$$\text{European efficiency index (EPI)} = \frac{\text{Final weight (kg)} \times \{100\text{-mortality rate (\%)}\}}{\text{FCR} \times \text{trail days}} \times 100$$

**Water consumption:** The flowmeter values of each repeat were recorded as the initial values before the start of the experiment and the flowmeter values were recorded at 24:00 on the 7th, 14th, 21st and 28th days of the experiment, respectively. The recorded values were subtracted from the initial values and then divided by the number of chickens in

Table 1: The experimental design and treatments

| | Experimental diet | | Ambient temperature (°C) | | | | |
|---|---|---|---|---|---|---|---|
| Items | 0-7 day | 8-28 day | 0-4 day | 5-7 day | 8-14 day | 15-28 day | Relative humidity (%) |
| NHC | | Basal diet, DLys 1.22% | 33-32 | 32-30 | 30-28 | 26 | 60-70 |
| HC | Basal diet, Dlys 1.12% | Basal diet, Dlys 1.12% | | | | | |
| HLA | | High AA diet, Dlys 1.22% | 19:00-7:00, 35°C, 19:00-7:00, 30°C | | | | 70-80 |
| HHA | | Low AA diet, Dlys 1.02% | | | | | |

NHC: Non heat stress control group, HC: Heat stress control group, HLC: Heat stress low amino acid group, HHA: Heat stress high amino acid group, the same below table

Table 2: Balanced digestible amino acid ratios of COBB 500

| Phases | Lys | Met | Met+Cys | Try | Thr | Arg | Val | Ile |
|---|---|---|---|---|---|---|---|---|
| Starter (0-8 day) | 100 | 38 | 75 | 16 | 68 | 108 | 76 | 64 |
| Grower-finisher (9-28 day) | 100 | 40-41 | 76-77 | 16-18 | 67-66 | 108-109 | 76 | 64-65 |

each stage to calculate the average drinking water volume per chicken. Additionally, feed consumption during the same period was recorded and the water-to-feed ratio (WFR) was calculated.

**Statistical analysis:** Excel 2021 was used for preliminary data sorting. Data were analyzed using the general linear model procedure of SAS 9.4[11] (SAS Institute, Cary, NC, USA) in a completely randomized design. If a statistically significant difference was found ($p<0.05$), a Fisher's least significant difference (LSD) test was used to test the pairwise differences between the treatment means. All data were expressed as "mean±standard deviation".

## RESULTS

**Effects of different dietary amino acid levels on growth performance of broilers under heat stress:** The growth performance results of different experimental groups are presented in Table 4. There were no significant differences in initial weight and weight at 7 days of age among all groups ($p>0.05$) and no significant differences in ADG, ADFI and FCR among all groups from 1-7 days ($p>0.05$). The final weight at 28 days of age was significantly different among the groups ($p<0.05$). The NHC group was significantly higher than the other three heat stress treatment groups ($p<0.05$), while the HLA group in the heat stress treatment groups was

Table 3: Dietary ingredients and nutrient composition of the experimental diets (as-fed basis)

| | 1-7 day | | 8-28 day | |
|---|---|---|---|---|
| Ingredients (kg $t^{-1}$) | Basal diet (DLys 0.22%) | Low AA diet (DLys 1.07%) | Basal diet (DLys 1.12%) | High AA diet (DLys 1.18%) |
| Corn | 268.0 | 224.8 | 212.0 | 198.9 |
| Wheat | 260.0 | 300 | 300 | 300 |
| Refined palm oil | 32.0 | 38 | 40 | 42 |
| Soybean meal, 46% | 290.0 | 260 | 270 | 280 |
| Rice bran | 50.0 | 90 | 90 | 90 |
| Meat and bone meal, 50% | 65.0 | 70 | 70 | 70 |
| Fish meal | 15.0 | | | |
| Lines tone | 1.0 | 1 | 1 | 1 |
| Salt | 3.5 | 3.5 | 3.5 | 3.5 |
| Choline chloride, 60% | 1.0 | 1 | 1 | 1 |
| D,L-methionine,99% | 3.1 | 2.1 | 2.4 | 2.8 |
| L-lysine HCL,98% | 2.6 | 1.3 | 1.7 | 2.1 |
| L-threonine, 99% | 1.2 | 0.5 | 0.6 | 0.9 |
| Bacillus subtilis | 0.1 | 0.1 | 0.1 | 0.1 |
| Tributyrin | 0.5 | 0.5 | 0.5 | 0.5 |
| Turmeric powder | 2.0 | 2 | 2 | 2 |
| Sodium humate | 0.8 | 1 | 1 | 1 |
| Enzyme[1] | 0.6 | 0.6 | 0.6 | 0.6 |
| Premix[2] | 3.6 | 3.6 | 3.6 | 3.6 |
| Total | 1000.00 | 1000.00 | 1000.00 | 1000.00 |
| **Calculated nutrient levels** | | | | |
| Dry matter (%) | 89.52 | 89.21 | 89.27 | 89.68 |
| ME/MJ $kg^{-1}$ | 12.55 | 12.65 | 12.65 | 12.65 |
| Crude protein (%) | 23.50 | 21.89 | 22.3 | 22.73 |
| Calcium (%) | 0.95 | 0.91 | 0.92 | 0.92 |
| Total phosphorus (%) | 0.84 | 0.83 | 0.83 | 0.83 |
| Available phosphorus (%) | 0.48 | 0.46 | 0.46 | 0.46 |
| Digestible lysine (%) | 1.22 | 1.02 | 1.12 | 1.22 |
| Digestible methionine (%) | 0.61 | 0.51 | 0.54 | 0.57 |
| Dmet (%)+DCys (%) | 0.91 | 0.79 | 0.84 | 0.93 |
| Digestible threonine (%) | 0.83 | 0.70 | 0.76 | 0.82 |
| Digestible tryptophan (%) | 0.21 | 0.2 | 0.21 | 0.22 |
| Digestible arginine (%) | 1.36 | 1.31 | 1.34 | 1.37 |
| Digestibel valine (%) | 1.02 | 0.99 | 1 | 1.02 |
| Digestible Isoleucine (%) | 0.82 | 0.78 | 0.8 | 0.82 |

[1]Complex enzyme preparation: 0.6 kg $t^{-1}$ contains 150 g $t^{-1}$ high temperature resistant phytase, 150 g $^{-1}$ t xylanase, 150 g $t^{-1}$ mannanase and 150 g $t^{-1}$ high temperature resistant Lipase, [2]Premix supplied per kg of diet: vitamin A:10000 IU, Vitamin D: 5 MIU, Vitamin E: 100 mg, Vitamin K: 3 mg, Riboflavin: 10 mg, Nicotinic acid: 55 mg, Calcium pantothenic acid: 20 mg, Folic acid: 2 mg, Thiamine: 3 mg, Riboflavin: 10 mg, Biotin: 0.3 mg, Pyridoxine: 5 mg, Cobalamin: 25 mg, Manganese: 100 mg, Iron: 40 mg, Zinc: 100 mg, Copper: 15 mg, Iodine: 1 mg and Selenium: 0.4 mg

Table 4: Effects of different dietary amino acid levels on growth performance of broilers

| Growth performance | | NHC | HC | HLA | HHA |
|---|---|---|---|---|---|
| Average body weight (g) | 1 day | 44.600±0.6 | 44.800±0.6 | 44.600±0.5 | 44.500±0.7 |
| | 7 day | 216.300±4.8 | 212.400±3.5 | 212.800±2.8 | 214.100±4.1 |
| | 28 day | 1735.900±29.4ᵃ | 1619.800±23.6ᶜ | 1672.100±20.1ᵇ | 1509.900±22.4ᵈ |
| Average daily gain (ADG, g/day) | 1-7 day | 24.500±0.3 | 23.900±0.3 | 24.000±0.4 | 24.200±0.3 |
| | 8-28 day | 72.400±4.6ᵃ | 67.000±1.6ᶜ | 69.500±1.9ᵇ | 61.700±1.8ᵈ |
| | 1-28 day | 60.400±2.7ᵃ | 56.300±1.5ᵇ | 58.100±2.1ᵇ | 52.300±2.4ᶜ |
| Average daily feed intake (ADFI, g/day) | 1-7 day | 24.280±0.3 | 23.950±0.3 | 23.970±0.4 | 24.020±0.7 |
| | 8-28 day | 111.310±3.5ᵃ | 106.330±2.7ᵇ | 108.520±1.8ᵇ | 99.120±1.4ᶜ |
| | 1-28 day | 91.130±3.3ᵃ | 87.350±2.2ᵇ | 88.980±1.5ᵇ | 81.970±1.1ᶜ |
| FCR | 1-7 day | 0.991±0.008 | 1.003±0.014 | 0.999±0.009 | 0.997±0.011 |
| | 8-28 day | 1.540±0.05ᵇ | 1.590±0.04ᵃ | 1.560±0.02ᵃᵇ | 1.610±0.07ᵃ |
| | 1-28 day | 1.470±0.02ᶜ | 1.510±0.20ᵃ | 1.490±0.03ᵃᵇ | 1.520±0.04ᵃ |

Mean values with different superscripts are significantly different (p<0.05). While the same or no letters indicate non significant differences (P>0.05)

Table 5: Effects of different dietary amino acid levels on mortality and EPI of broilers

| | Mortality (%) | | EPI | |
|---|---|---|---|---|
| Treatments | 1-7 day | 1-28 day | 1-7 day | 1-28 day |
| NHC | 1.73±0.17 | 5.48±0.19ᵈ | 306.41±9.3 | 398.6±12.3ᵃ |
| HC | 1.94±0.12 | 8.42±0.21ᵇ | 296.60±6.9 | 350.9±9.7ᶜ |
| HLA | 1.98±0.15 | 6.11±0.23ᶜ | 298.20±8.3 | 376.3±11.2ᵇ |
| HHA | 2.04±0.21 | 9.73±0.28ᵃ | 300.50±6.4 | 320.3±8.3ᵈ |

Mean values with different superscripts are significantly different (p<0.05). While the same or no letters indicate non significant differences (P>0.05)

significantly higher than the HC group and HHA group (p<0.05). From 8-28 days, the ADG and ADFI of the NHC group were significantly higher than those of each heat stress treatment group (p<0.05) and in heat stress treatment group, the ADG in HLA group was higher than that in HC and HHA groups and the ADG and ADFI in HHA group were significantly lower than those in other groups (p<0.05). During the whole period (1-28 day), the ADG and ADFI of NHC were significantly higher than those of each heat stress treatment group (p<0.05) and the FCR was significantly lower than that in HC and HHA (p<0.05) but not significantly compared to HLC. The ADG and ADFI of the HHA group were significantly lower than those of the other treatment groups (p<0.05). No significant differences were observed in ADG and ADFI between the HLA and HC group (p>0.05), although the values were slightly higher.

The above results indicated that heat stress did not affect the daily gain and feed intake of 1-7 days old COBB broilers but significantly decreased growth performance of 8-28 days old COBB broilers. Reducing the level of digestible amino acids in the diet can improve feed intake and daily gain, reduce FCR and alleviate heat stress of 8-28 days old COBB broilers. However, there is still a disparity in growth performance compared to the non-heat stress group.

**Effects of different dietary amino acid levels on mortality and EPI of broilers under heat stress:** In the first week, there was no significant difference in the mortality rate and EPI among the groups (p>0.05) but numerically, the NHC group had a lower mortality rate and a higher EPI (Table 5). During the whole period (1-28 day), the mortality rate of NHC group was significantly lower and EPI was significantly higher than the other three heat stress groups (p<0.05). In heat stress treatment groups, the mortality rate of HLA group was significantly lower and EPI was significantly higher than that of NHC and HHA groups (p<0.05), the mortality rate of HHA group was significantly higher and EPI was significantly lower than that of other groups (p<0.05). The above results showed that heat stress had little effect on the mortality rate of broilers aged 0-7 days but led to a significant increase in the mortality rate of broilers aged 8-28 days. Simultaneously, a high amino acid level can elevate the mortality rate of broilers under heat stress. In contrast, feeding diets with a low amino acid level can decrease the death rate of broilers caused by heat stress, thereby enhancing economic benefits.

**Effects of different dietary amino acid levels on water to feed ratio (WFR) of broilers under heat stress:** The average water consumption results of the treatment groups were presented in Table 6. There were no significant differences in the average daily water consumption and WFR among all groups at first week (0-7 day) (p>0.05). From 8-28 days, the average daily water consumption and WFR of HHA group were significantly higher than those of other groups and the average daily water consumption of NHC group was significantly lower than those of other groups (p<0.05). During

Table 6: Effects of different dietary amino acid levels on Water consumption of broilers

| | Average water consumption (mL/day) | | | Water to feed ratio | | |
|---|---|---|---|---|---|---|
| Treatments | 1-7 day | 8-28 day | 1-28 day | 1-7 day | 8-28 day | 1-28 day |
| NHC | 48.62±0.7 | 190.22±3.6[c] | 154.82±3.4[c] | 1.97±0.03 | 2.09±0.21[c] | 2.05±0.19[c] |
| HC | 48.03±0.5 | 339.40±6.9[b] | 266.56±6.2[b] | 1.99±0.05 | 3.58±0.18[b] | 3.46±0.13[b] |
| HLA | 48.01±0.4 | 323.70±7.6[b] | 254.78±7.1[b] | 2.04±0.09 | 3.20±0.11[b] | 3.11±0.10[b] |
| HHA | 48.31±0.5 | 374.49±9.4[a] | 292.95±9.0[a] | 2.01±0.10 | 3.89±0.22[a] | 3.74±0.21[a] |

Mean values with different superscripts are significantly different (p<0.05). while the same or no letters indicate non significant differences (P>0.05)

the whole period (1-28 day), the average daily water consumption (292.95 mL/day) and WFR (3.74 mL/day) of HHA in experimental groups were significantly higher than those of the other groups (p<0.05) and the average water consumption (154.82 mL/day) and WFR (2.05 mL/day) in NHC group were significantly lower than those of the other groups (p<0.05). There were no significant differences in average daily water consumption and WFR between HC and HLA groups (p>0.05).

## DISCUSSION

**Effects of heat stress on growth performance of broilers:** To date, numerous studies have investigated the impact of heat stress on the growth performance of poultry. In general, heat stress primarily impacts the production performance of animals by decreasing feed intake and nutrient metabolism. Egg-laying birds experience a decline in egg production rate and weight, along with reduced eggshell quality, while slowing down growth and increasing mortality rate in meat birds[12]. For poultry, previous studies had demonstrated that, when the ambient temperature was 21-30°C, feed intake decreased by 1.5% for every 1°C increase in temperature. Furthermore, when the ambient temperature exceeded 32°C, feed intake decreased by 4.6% for every 1°C increase in ambient temperature[13]. Additionally, under normal conditions, the water consumption of chickens was approximately twice that of their feed intake but if the house temperature exceeds the optimal temperature, broilers can increase their water intake within a few hours, with the maximum reaching up to 9 times of feed intake[14].

In this experiment, the temperature and humidity of the chicken house were controlled to simulate the high-temperature and high-humidity environment in Indonesia. The heat stress treatment groups were exposed to the heat stress environment from the first day of check-in until the 28th day of age. Based on the overall results of this experiment, under the condition of feeding the same diet, heat stress markedly decreased the final weight of 28-day-old COBB broilers and also led to a reduction in the average daily feed intake and daily gain of broilers aged 1-28 days, while the FCR significantly increased. These findings are consistent with previous studies conducted by Niu *et al.*[15] and Awad *et al.*[16]. When broiler chickens experience heat stress, their excitement of their feeding center is partially inhibited, resulting in decreased appetite and feed intake and the body utilizes its stored nutrients in response to heat stress. Moreover, heat stress induces intestinal dysfunction, diminishing the digestion and absorption of chyme in the intestine, ultimately leading to slow growth and an increased in feed-to-meat ratio in broilers[12]. In addition, the results of this experiment revealed that compared with the non-heat stress group, water consumption and feed-to-water ratio were significantly increased, the mortality rate increased by nearly 3% and the EPI decreased by 48. Furthermore, the feed-to-water ratio can serve as an indicator to evaluate whether broilers are under heat stress. During heat stress, broilers dissipate heat and lower body temperature by increasing water intake. However, high feed-to-water ratios can easily result in increased fecal moisture content and wet litter in the chicken house, leading to diseases such as coccidia and intestinal enteritis, which will not only affects the growth performance of broilers but also impacts their health status, leading to an increase in mortality rates and economic losses[17].

**Effect of different dietary amino acid levels on growth performance of broiler under heat stress:** The reports lack consensus on whether adjusting the dietary digestible amino acid level can enhance the growth performance of broiler chickens under heat stress. In broilers under heat stress, there is a common belief that, adding fat to the feed alleviate heat stress due to reduced feed intake and inadequate energy and protein intake. However, as compared to fat, protein generates more heat during metabolism. Although, supplementing protein in the diet can compensate for the reduced protein intake due to reduced feed intake, the protein metabolism process simultaneously increases body heat production, exacerbating the impact of heat stress on broilers[8]. Furthermore, the metabolism of excess protein into uric acid further consumes energy, adversely affecting growth performance[18]. Therefore, authors of previous studies agree that supplementing oil, reducing dietary crude protein content and incorporating limiting amino acids to achieve amino acid balance can mitigate heat stress and enhance the growth performance of broiler chickens under heat stress[19,20].

In this experiment, although the amino acid concentration in low and high amino acid diets varied, the balance of digestible amino acids in the diet was achieved by supplementing exogenous limiting amino acids. The results of the present study indicate that increased digestible amino acid levels in diets may exacerbate heat stress. In the high amino acid heat stress group, the feed intake and the final weight at 28 days of age significantly decreased, the mortality rate was increased by 17% compared with the heat stress control group and the profit index (European benefit index) was decreased by nearly 31. This result suggests that feeding diets with high concentrations of amino acids under heat stress increased the metabolic burden and heat production of amino acids in broilers. It not only causes a negative energy balance and stunted growth in broilers but also increases the burden on their internal organs, especially the intestines, liver and kidneys, resulting in an increased risk of sudden death[19]. Moreover, high temperature reduces the availability of feed source Arginine (Arg), making the actual digestible Arg level to fall significantly below the formula design value, thus showing Arg deficiency, resulting in lower feed conversion efficiency for broilers[21]. Additionally, diets with high Lys levels heighten the antagonistic effect between Lys and Arg, further decreasing the digestibility of arginine in diets[7]. In the absence of exogenous addition of Arg, the growth performance of broilers will be significantly affected, which may also be another reason for the poor growth performance observed in the high amino acid group under heat stress.

Furthermore, early studies have shown that high temperature can markedly alter the protein or amino acid requirements of broilers[3,8]. Under heat stress, protein synthesis decreases and decomposition increases and this decrease in protein synthesis cannot be compensated by elevating dietary protein levels due to the high heat production of protein[22]. The current results indicate that the optimal amino acid level of broilers under a normal temperature may not lead to the best growth performance under heat stress environment. Reducing the digestible amino acid level can promote the feed intake of broilers under heat stress, improve growth performance and reduce the mortality rate of broilers. This result is similar to previous studies, which suggested that reducing protein or Lys concentration during heat stress can diminish body heat production caused by amino acid metabolism, thereby alleviating heat stress[7,8]. Moreover, the results of this experiment also demonstrated that the digestible amino acid requirement of broilers under heat stress is lower than that under normal ambient temperature. However, the optimal digestible amino acid level for broiler chickens under heat stress cannot be determined in this experiment. Simultaneously, further research is needed to explore the mechanism by which low digestible amino acid levels enhance the growth performance of heat-stressed broiler chickens.

## CONCLUSION

Heat stress reduced the feed intake and daily weight gain, increased the water-to-feed ratio and the mortality rate, ultimately reduced the economic benefit of 8-28 days old COBB broilers. Under heat stress, diets with high digestible amino acid levels could exacerbate the effect of heat stress but using diets with low levels of digestible amino acid in amino acid balance could enhance the growth performance, reduce the mortality rate of COBB broilers aged 8-28 days and play a role in alleviating heat stress.

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