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Response of broilers to reduced-protein diets under heat stress conditions

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Decreasing heat increment through lowering crude protein (CP) level *via* supplemental amino acids (AA) have long been suggested as a nutritional practice to alleviate negative heat stress effects on broiler performance. However, there is a considerable body of inconclusive reports on optimisation of such practices, whereby bird performance remains unchanged. The exact mechanism underlying the impaired growth performance in birds fed with reduced-CP diets is not clear yet. Furthermore, adding the environmental temperature factor to the situation may complicate the solution. To date, there is no agreement on the extent of reducing CP level *via* AA supplementation so that growth performance remains unaffected. Evidence suggests that dietary CP could be reduced safely by 2.3% *via* essential AA supplementation during later ages, when birds are exposed to an average daily ambient temperature of ≤27.3°C. When Gly was added (a non-essential AA source), the margin of CP reduction could be increased to 5.1% without compromising the growth of broilers subjected to cyclic heat stress. Nonetheless, feeding broilers with a similar Gly-fortified, reduced protein diet failed to support optimal performance under hot and humid tropical climates in 1-21-d-old broilers and had a major impact on growth in broilers reared at 34°C. Regardless of supplemental AA composition or the level of CP reduction, the performance of broilers was negatively affected when birds were subjected to chronic heat stress conditions (≥30°C). These discrepancies can be attributed to a wide range of confounding factors, such as the extent of lowering CP level, types of AA used, age and environmental conditions. Accordingly, the addition of Gly may represent a good approach for reducing dietary CP levels for broilers raised under elevated ambient temperature. Reducing dietary CP is recommended when birds are exposed to moderate but not chronic heat stress conditions.

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Introduction

Heat stress is a common problem in poultry production, particularly in tropical and subtropical regions, and causes major economic losses annually. The adverse effects of heat stress on the broiler growth rate, feed intake and feed efficiency are well documented (Daghir, 2008; Lara and Rostagno, 2013). Heat-stressed birds exhibit several physiological responses to maintain thermoregulation, thereby decreasing body temperature.

Research into the use of supplemental amino acids (AA) to reduce the crude protein (CP) content of diets for maintaining optimal performance has been the subject of extensive studies. This is of interest because reducing dietary protein can minimise the environmental pollution by decreasing nitrogen (N) excretion. Besides, reducing dietary CP has a particular advantage for producers in tropical regions. Given the fact that heat increment of CP is the highest, compared to fat and carbohydrates (Musharaf and Latshaw, 1999), it has been proposed that the adverse effects of heat stress on poultry performance can be alleviated by reducing CP (Waldroup, 1982; Zaman *et al.*, 2008).

The effects and limits for reducing dietary CP through AA fortification on growth performance of broilers have been reviewed by Aftab *et al.* (2006), but no attention was given to the effects of reduced-CP diets on heat-stressed broilers. Using growth performance as the main criteria; this paper reviews the responses of broiler chickens to reduced-CP diets under heat stress conditions. Factors affecting the limit(s) for dietary CP reduction without compromising performance of broilers reared at high environmental temperatures are reviewed in this paper.

Protein and amino acids requirements of broiler chickens

Amino acids, obtained from dietary protein sources, are used by poultry to meet a variety of functions and can be divided into two main categories. The AAs which cannot be synthesised are classified as essential amino acids (EAA), and those that can be synthesised in the body are classified as non-essential amino acids (NEAA; NRC, 1994). However, there are a few AAs which are not synthesised in the body fast enough to meet maximum growth requirements. Therefore, it is necessary that these AAs be supplied in the diet. The nutritional classification of amino acids for poultry is presented in *Table 1*.

Broiler chickens do not require an exact level of CP; rather, they require an exact and balanced quantity of EAA, and adequate protein, either in the form of ready to use NEAA or as N for NEAA synthesis (NRC, 1994). However, broiler requirements for AA is affected by many factors, including high ambient temperature, gender, age, strain, and some diet-related factors, such as metabolisable energy (ME), AA imbalance, the effects of vitamins and coccidiostats, and amino acids antagonistic relationships (D'Mello, 2003).

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Table 1 Nutritional classification of amino acids for poultry.

Not synthesised in chickens (Essential, indispensable)	Synthesised from limited substrates*	Readily synthesised from simple substrates (Non-essential, dispensable)
Arginine	Tyrosine	Alanine
Lysine	Cysteine	Aspartic acid
Histidine	Hydroxylysine	Asparagine
Leucine		Glutamic acid
Isoleucine		Glutamine
Valine		Hydroxyproline
Methionine		Gly
Threonine		Serine
Tryptophan		Proline
Phenylalanine		

*Tyrosine is synthesised from phenylalanine, Cysteine from methionine, and hydroxylysine from lysine.
Source: Scott *et al.* (1982)

Despite much research to investigate dietary protein requirements of birds kept under high ambient temperatures, very little, and often conflicting data, on amino acids requirements under such conditions are available. Some research has suggested unchanged lysine (Sinurat and Balnave, 1985; Mendes *et al.*, 1997), threonine (Shan *et al.*, 2002), and tryptophan (Ojano-Dirain and Waldroup, 2002; Shan *et al.*, 2002) requirements at high temperatures. However, other research recommended higher lysine (Han and Baker, 1993; Ojano-Dirain and Waldroup, 2002) and methionine (Ojano-Dirain and Waldroup, 2002) requirements for heat-stressed birds. Conversely, some published data suggested decreased lysine (Waldroup, 1982; Corzo *et al.*, 2003), methionine (Balnave and Oliva, 1990) and arginine (Chamruspollert *et al.*, 2004). These conflicting results could be attributed to the wide differences in the duration and severity of heat exposure among these studies.

In terms of gender, some studies have suggested that males require greater quantities of AA than females at a similar age. It has been indicated that lysine (Han and Baker, 1993; 1994; Dozier *et al.*, 2008; 2009), methionine (Thomas *et al.*, 1986), threonine (Thomas *et al.*, 1987; Dozier *et al.*, 2001), arginine (Kessler and Thomas, 1976), and tryptophan (Hunchar and Thomas, 1976) requirements are higher for male broilers than for females. This was attributed to the higher protein and less fat deposition in male weight gain (Han and Baker, 1994).

It has been consistently reported that dietary requirements for all AA and CP decrease with the age of the chicken (NRC, 1994). The NRC (1994) suggested AA requirements for three age periods 0-3 (starter), 3-6 (grower/finisher), and 6-8 (finisher) weeks of age (*Table 2*). However, most of the present commercial broiler chicken breeders, such as Cobb 500, Ross 308, Arbor Acres and Hubbard, suggest AA specifications for shorter age periods (*e.g.*, d 0- 10, 11- 24, 25- 39, 40- 51, and > 52 d) for more AAs utilisation efficiency.

In terms of broiler strains, significant improvements in broiler performance and carcass composition have been achieved as a result of genetic selection, and CP and AA requirements of these new genotypes have changed accordingly. The AA requirements of today's broiler chickens are relatively higher than those suggested by the NRC (1994). By comparing the recommended AA specifications for today's broiler chicken strains, differences can be observed (*Table 3*). It is worth mentioning that suggested AA specifications for all strains are set to support an ideal amino acid profile, where the requirement of the main amino acids that may be limited in broiler feeds are calculated, and with lysine used as the reference AA to which ratios are set for the other AAs. Unlike

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NRC (1994), suggested ratios for the ideal AA profile of these broiler strains are based on digestible AA.

Table 2 Protein and amino acids requirements of broilers (%).

Nutrient	0-3 weeks	3-6 weeks	6-8 weeks
Crude protein[#]	23	20	18
Arginine	1.25	1.10	1.00
Gly + Serine	1.25	1.14	0.97
Histidine	0.35	0.32	0.27
Isoleucine	0.80	0.73	0.62
Leucine	1.20	1.09	0.93
Lysine	1.10	1.00	0.85
Methionine	0.50	0.38	0.32
Methionine + Cysteine	0.90	0.72	0.60
Phenylalanine	0.72	0.65	0.56
Phenylalanine + Tyrosine	1.34	1.22	1.04
Proline	0.60	0.55	0.46
Threonine	0.80	0.74	0.68
Tryptophan	0.20	0.18	0.16
Valine	0.90	0.82	0.70

[#] Broiler chickens do not have a requirement for crude protein per se. There should be sufficient crude protein to ensure an adequate nitrogen supply for the synthesis of non-essential amino acids, however. Suggested requirements for crude protein are typical of those derived with corn-soybean meal diets, and levels can be reduced when synthetic amino acids are used.
Source: NRC (1994)

Protein requirements of broiler chickens under hot climate

Determining the protein requirements of broiler chickens at high ambient temperatures has been a field of research for many years. However, findings have been inconsistent and different recommendations have been made. The first was to increase dietary protein, to compensate for reduced protein intake as a consequence of decreased feed intake associated with heat stress (Temim *et al.*, 1999; 2000; Gonzalez-Esquerra and Leeson, 2005). Temim *et al.* (1999) reported significantly higher weight gain and better feed efficiency when heat-stressed broiler chickens were fed diets higher in CP (25% *vs*. 19.6%) from 4-6 weeks of age. Gonzalez-Esquerra and Leeson (2005) reported improvements in broiler performance when high crude protein diets were fed from d 21 to 42 at 27.3°C and 31.4°C housing temperatures. Conversely, excess dietary protein reduced the growth rate of commercial, fast-growing broiler chickens raised at a constant 32°C from 4-8 weeks of age (Cahaner *et al.*, 1995). Cheng *et al.* (1997) noted adverse effects of high protein diets (22% or 24% CP) on growth performance in 3-6-week old heat-stressed broilers. Similarly, Cheng *et al.* (1999) found that increasing CP (22 *vs*. 20% CP) and AA (110%) concentrations to compensate for reduced feed intake when broilers were reared under constant (32.2°C) or cyclic (26.6°C for 16 h to 35°C 8 h) heat stress conditions, negatively affected growth performance. Suppressed growth performance when feeding excess dietary protein diets under heat stress conditions can be attributed to higher metabolic heat production because protein has the highest heat increment during metabolism or catabolism compared to other nutrients (Musharaf and Latshaw, 1999). Basically, any increase in dietary CP will be followed by an increase in nitrogen excretion, which is energetically expensive (Musharaf and Latshaw, 1999).

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Accordingly, the poorer performance seen when broilers were fed increased-CP diets under heat stress conditions could be explained by the higher heat production (associated with protein synthesis and from nitrogen excretion) as well as lower energetic efficiency.

Therefore, dietary CP reduction, while maintaining AA within the recommended requirements, has long been suggested as the best strategy (Waldroup, 1982; Austic, 1985; Teeter, 1994). Later, this approach was supported by the advantages of reducing feed cost and nitrogen excretion.

Effects of reduced-CP diets on the performance of broilers reared at elevated environmental temperatures

The availability of synthetic AA, accompanied by the establishment of the ideal protein concept (Baker, 1997), have contributed to a decrease in dietary CP in broiler diets. In general, there is consensus that synthetic AA in broiler diets can replace CP to a certain level, and any reduction beyond that level has been shown to adversely affect the growth performance of broiler chickens, even when the EAA requirements are met. However, there is considerable disagreement on the exact level of appropriate reduction. It is very difficult to cover all studies on broilers' growth performance in response to feeding reduced-CP under heat stress in a review of studies. Hence, in this review, only selected studies have been reviewed and summarised in chronological order (*Table 4*).

Early research to evaluate the influence of reduced-CP-diet on broiler performance under heat stress conditions was carried out by Alleman and Leclercq (1997). In their study, heat-stressed (32°C constant heat stress) broilers were fed a diet reduced by 4% CP (16% *vs.* 20% CP), fortified with methionine, lysine, threonine, valine and arginine in order to meet requirements from 23 to 44 d of age. The reduced-CP diet significantly increased FCR (2.413 *vs.* 2.194) and decreased body weight gain (939 *vs.* 1118 g) compared to the control group.

Two years later, Cheng *et al.* (1999) again applied a 4% reduction in dietary CP (16% *vs.* 20% CP) which negatively affected the performance of broilers under constant (32.2°C) and cyclic (26.6°C for 16 h to 35°C 8 h) heat stress conditions from 21 to 49 d of age. Under constant heat stress conditions, the reduced-CP diet resulted in significantly lower gain/feed ratio (0.315 *vs.* 0.380) and weight gain (1280 *vs.* 945 g) compared to the control group. Under the cyclic heat stress conditions, the reduced-CP diet significantly decreased feed intake (2368 *vs.* 3023 g/bird) and weight gain (1045 *vs.* 1071 g) compared to the control group.

Faria Filho *et al.* (2005) evaluated the performance of 7 to 21-d old broilers fed a 3% reduced CP diet (18.5 *vs.* 21.5% CP) fortified with all EAA except Phe, Leu and His, and maintained under constant heat stress conditions of 33°C. The birds fed the reduced-CP diet showed significantly higher FCR (1.48 *vs.* 1.40) and lower weight gain (623 *vs.* 663 g) compared to birds fed the 21.5% CP control diet.

At almost the same time, Gonzalez-Esquerra and Leeson (2005) evaluated the effects of reducing dietary CP from 20 to 18% CP on the performance of broilers reared under acute (constant 27.3°C) or chronic (constant 31.4°C) heat stress conditions from 21 to 42 d of age. The authors reported no effect on body weight gain, feed intake or feed:gain ratio in birds reared at 27.3°C. However, the reduced-CP diet resulted in significantly higher feed:gain ratio (2.40 *vs.* 2.26) in broilers reared at 31.4°C.

A study by Faria Filho *et al.* (2007) evaluated the influence of a 3% reduction in dietary CP (from 20.0 to 17.0%) *via* supplementation with Met, Lys, Thr, Val, Arg, Trp and Ile in broilers kept under constant heat stress conditions (32.2°C) from 21 to 42 d of

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age. The reduction in CP negatively affected the growth performance of broilers by increasing the FCR by 9.0% (1.93 *vs.* 1.77).

Zaman *et al.* (2008) conducted an experiment to evaluate the performance of broiler chickens reared in a hot climate and fed diets varying in CP (20.8 *vs.* 18.8% CP) and metabolisable energy from seven to 28 d of age. When the birds were fed the standard energy level (12.55 MJ/kg), a 2% reduction in CP did not affect weight gain, feed intake or feed:gain ratio of broiler chickens. Similarly, Laudadio *et al.* (2012) reported similar growth performance when broilers were fed a reduced-CP diet (2% reduction; from 20.5 to 18.5% CP) under summer environmental conditions from 14 to 42 d of age. In both the Zaman *et al.* (2008) and Laudadio *et al.* (2012) studies, reduced-CP diets were fortified with the limiting amino acids Met, Lys and Thr to meet requirements.

Awad *et al.* (2014a) used 1 to 21-d-old broiler chickens to investigate the effect of feeding different ideal EAA supplemented, reduced-CP diets (19.2 or 17.9% CP) under tropical climates, *i.e.* a hot and humid environment with maximum (at least 6 h per day) and minimum temperatures of 35°C and 26°C, respectively. Compared to a control group of broilers fed 20.6% CP, the reduction in CP by 1.4% did not affect performance of broilers. However, the reduction in CP by 2.7% resulted in significantly lower weight gain (707 *vs.* 778 g) and higher FCR (1.48 *vs.* 1.39) compared to the 20.6% CP group.

In a different study in a hot tropical environment maximum (for at least for 6 h per day) and with minimum temperatures of 34.5°C and 26°C, respectively, Awad *et al.* (2015) evaluated the effect of 5.5% reduction in CP, from 22.3 to 16.8% C,P fortified with all EAA to meet their requirements plus the NEAA Gly, on the growth performance of broilers from 1 to 21 d of age. The authors found that feeding the Gly fortified, reduced-CP diet resulted in significantly lower feed intake (1119 *vs.* 1186 g/bird), poorer body weight (844 *vs.* 923 g) and higher FCR (1.41 *vs.* 1.35) compared to the 22.2% CP diet.

A study by Liu *et al.* (2016) examined the effect of a 3.3% reduction in CP (from 18.2 to 14.9% CP) supplemented with Met, Lys, Thr and Trp on the growth performance of broiler chickens reared under cyclic heat stress conditions (32°C for 4 h , 28°C for 16 h to 23°C for 4 h) from 28 to 42 d of age. The authors reported a that, when birds were fed the reduced-CP diet, FCR was significantly higher (2.30 *vs.* 2.12) compared to their counterparts fed the 18.2% CP diet.

Considering the previously available data, Awad *et al.* (2017) evaluated the effect of a 5% reduction in dietary CP in the starter (22.3 *vs.* 17.3% CP) and finisher (19.4 *vs.* 14.5% CP) diets with EAA fortification plus Gly on growth performance of male and female broiler chickens reared under a hot tropical environment (maximum and minimum temperatures of 35.7°C and 25.8°C, respectively). Regardless of gender, feeding the reduced-CP diet negatively affected growth performance during both the starter (d 1 to 21) and overall (d 1 to 42) periods. During the starter period, the reduced-CP diet resulted in significantly lower feed intake (1075 *vs.* 1166 g/bird) and weight gain (754 *vs.* 846 g) and higher FCR (1.43 *vs.* 1.38) compared to control group. During the overall period, the group fed reduced-CP diet showed significantly lower feed intake (3768 *vs.* 4073 g/bird) and weight gain (2091 *vs.* 2251 g) compared to the control group.

Recently, Zulkifli *et al.* (2018) investigated the effect of reducing dietary CP from 19.1 to 16.2% CP (-2.9 %) with supplemental Met, Lys, Thr and Trp on growth performance of 22 to35 d old broiler chickens reared under cyclic heat stress conditions (33°C for 6 h to 23°C for 18 h). The reduced-CP diet adversely affected performance of broilers by significantly reducing weight gain (700 *vs.* 1055 g) and increasing FCR (2.03 *vs.* 1.79).

At the same time, Law *et al.* (2018) evaluated the influence of reducing dietary CP from 21.0 to 18.5% CP in the starter (d 1 to 21) diet and from 19.0 to 16.7% CP in the finisher (d 22 to 35) diet, with Met, Lys, Thr and Trp supplementation, with or without protease on growth performance of broiler chickens reared in a hot and humid tropical

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environment (at least 6 h of 36°C maximum temperature and 26°C minimum temperature). Birds fed the reduced-CP diet had significantly lower weight gain (1721 *vs.* 1894 g) and higher FCR (1.87 *vs.* 1.73) compared to their counterparts fed the control diet.

With this previous published data in mind, Awad *et al.* (2018) evaluated the effect of a 5.1% reduction in CP (19.4 *vs.* 14.3%) with supplementation with all EAA to meet their requirements plus Gly, on the performance of broiler chickens raised under cyclic (34°C for 7 h to 23°C for 17 h) or constant (34°C) heat stress conditions. The reduced-CP diet had no effect on growth performance of broilers under cyclic heat stress conditions. However, under constant heat stress conditions, feeding the reduced-CP diet resulted in significantly lower feed intake (1547 *vs.* 2158 g/bird), lower weight gain (584 *vs.* 975 g) and higher FCR (2.65 *vs.* 2.21) in comparison with those fed the control diet.

More recently, Law *et al.* (2019) investigated the effects of protease supplementation in a 2.3% reduced-CP diet (19 *vs.* 16.7% CP), supplemented with Met, Lys, Thr and Trp on performance of broiler chickens reared under cyclic heat stress conditions (34°C for 7 h to 23°C for 17 h) from 22 to 42 d of age. Regardless of protease supplementation, feeding birds with the reduced-CP diet did not impede growth performance of broilers compared to birds fed the control diet.

Factors affecting the response of broiler chickens to reduced-CP diets under heat stress conditions

Based on the findings from the above studies, it is complicated to compare the data dealing with feeding reduced-CP diets at high environmental temperatures, because numerous factors may be involved. These include energy level (Zaman *et al.*, 2008; Law *et al.*, 2019), the severity of heat exposure (Cheng *et al.*, 1999; Gonzalez-Esquerra and Leeson, 2005; Awad *et al.*, 2018), supplemental EAA compositions (Balnave *et al.*, 1999; Balnave and Barke, 2002) and supplemental NEAA (Awad *et al.*, 2014b). Other factors, such as protease supplementation (Law *et al.*, 2018; 2019) and sex (Awad *et al.*, 2017) must be considered when investigating the effect of feeding reduced-CP diets at elevated environmental temperatures, making it difficult to define an exact point for dietary CP reduction under such conditions. Three factors (the severity of heat exposure, supplemental AA compositions and age) which appeared to play a major part in determining the level of CP reduction in broiler diets under heat stress conditions will be further discussed.

SEVERITY OF HEAT EXPOSURE

In order to compare between the different environmental conditions of the selected studies, the average daily temperature of the different environments/heat challenge regimens were estimated (*Table 4*). The severity of heat stress conditions, in terms of intensity or duration of heat stress, seems to be the most influential factor on the response of heat-stressed broilers to reduced-CP diets. By comparing the effects of constant medium temperature (27.3°C) *vs.* constant high temperature (31.4°C) on the response of broilers fed a 2% reduced-CP diet, Gonzalez-Esquerra and Leeson (2005) found no negative impact of feeding the reduced-CP diet on growth performance in broilers reared at 27.3°C. However, feeding broilers with the same reduced-CP diet at 31.4°C led to inferior performance by increasing FCR by 6.2% compared with that of the control group, a negative response mainly attributed to the higher heat stress intensity. Similarly, Awad *et al.* (2018) compared the response of broilers being fed a Gly-fortified, 5.1% reduced protein diet under constant (34°C) and cyclic (average daily

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temperature of 26.2°C) heat stress conditions. The group of broilers fed the Gly-fortified reduced protein diet had similar feed intake, weight gain and FCR to those of the controls under the cyclic heat stress conditions. In contrast, feed intake, weight gain and FCR were negatively affected by 28.3, 40.1 and 16.6%, respectively when broilers were fed the Gly-fortified, reduced protein diet under constant heat stress conditions. By comparing the results of the above study under cyclic heat stress conditions with an earlier study from the same group, Awad *et al.* (2017) found that feeding broilers with a similar Gly-fortified reduced protein diet under hot and humid tropical climate conditions resulted in poorer performance compared to the control group. The discrepancy between these two studies could be attributed to the greater degree of heat that birds may have experienced (average daily temperature of 28.5°C) in the first study (Awad *et al.*, 2017) compared to the 26.2°C average daily temperature experienced by their counterparts under cyclic heat stress conditions in the second study (Awad *et al.*, 2018).

Findings from two recent studies by Law *et al.* (2018) and Law *et al.* (2019) provided evidence that the response of heat-stressed broilers to reduced-CP diets mainly depends on the severity of heat exposure. While the change in CP from 19 to 16.7% had no negative effect on growth performance under cyclic heat stress (average daily temperature of 26.2°C) conditions (Law *et al.*, 2019), feeding a similarly reduced-CP diet negatively affected the weight gain and FCR of broilers in a hot and humid tropical environment, where the estimated average daily temperature was at least 28.5°C (Law *et al.*, 2018). Altogether, the findings of the aforementioned studies indicated that broilers may respond positively to feeding a reduced-CP diet when birds are exposed to average daily ambient temperature of ≤27.3°C. This may explain the discrepancy between the results of previous studies on the response of broiler chickens to reduced-CP diets under high environmental temperatures. It can further explain the reported improvement in broiler chickens' performance when high crude protein diets were fed under chronic heat stress conditions (32°C), which led to a recommendation to increase dietary protein rather than feeding reduced-CP diets under such high environmental temperatures (Temim *et al.*, 1999; 2000).

SUPPLEMENTAL AMINO ACID COMPOSITIONS

Supplemental amino acid composition seems to be a major defining factor for the margin for CP reduction in broiler diets. This can generally be categorised into two groups. First, studies that supplemented their reduced-CP diets only with EAA. From the selected studies, CP can be reduced up to 2.3% when EAA supplementation is used, without any adverse effect on the performance of broilers reared under acute heat stress conditions (Gonzalez-Esquerra and Leeson, 2005; Zaman *et al.*, 2008; Laudadio *et al.*, 2012; Awad *et al.*, 2014a; Law *et al.*, 2019), Any reduction beyond this level has been shown to adversely affect performance (Alleman and Leclercq, 1997; Cheng *et al.*, 1999; Faria Filho *et al.*, 2007; Awad *et al.*, 2014a; Liu *et al.*, 2016; Zulkifli *et al.*, 2018), even when the requirements of all EAA are met (Awad *et al.*, 2014b). Secondly, some studies supplemented their reduced-CP diets with EAA and NEAA. By fortification with Gly (NEAA) to achieve Gly + serine levels similar to standard protein diets, and supplementation with all EAA to meet their minimum requirements, Awad *et al.* (2018) reported that CP could be reduced by 5.1% without affecting the growth performance of broilers subjected to cyclic heat stress conditions. Knowing the number of non-protein pathways that Gly participates in can further explain the reasons why it can potentially improve the performance of broilers (Akinde, 2014b). Gly is important for methionine recycling and cysteine biosynthesis, creatine synthesis, threonine catabolism and uric acid synthesis (Akinde, 2014a). Moreover, Gly represents the main part of the gut mucin glycoproteins (Moran, 2010). In a nutshell, Gly can

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promote the metabolic and nutritional efficiency of EAA as well as gut functionality and consequently growth performance. However, feeding such a fortified, reduced protein diet did not support growth equal to a standard protein diet in broilers kept under hot and humid tropical conditions (Awad *et al.*, 2017) and in birds exposed to 34°C constant heat stress (Awad *et al.*, 2018) and these results could be attributed to the greater degree of heat that birds may have experienced.

AGE

Broilers are more suitable heat stress during later ages. Accordingly, most of the studies evaluating the response of broiler chickens to reduced-CP diets were conducted during the grower or finisher periods. However, it is well known that broilers require higher dietary CP during early life. Available trials which evaluated the response of heat-stressed broilers to feeding such reduced-CP diets during both early and late ages showed that heat-stressed broilers were more sensitive to reduced-CP diets during early life. By comparing two different studies from the same laboratory using 7 to 21 d old (Faria Filho *et al.*, 2005) or 21 to 42 d old heat stressed broilers (Faria Filho *et al.*, 2007), reducing dietary CP by 3% negatively affected the body weight gain of broilers during early age (Faria Filho *et al.*, 2005) without affecting the weight gain of their counterparts fed a 3% reduced-CP diet during the later age (Faria Filho *et al.*, 2007). In the study of Awad *et al.* (2017), feeding the Gly-fortified, reduced protein diet negatively affected the feed intake, weight gain and FCR by 7.8, 10.9 and 3.6%, respectively, from 1 to 21 d of age (starter phase). However, this effect was lesser in severity when considering the overall experimental period, as feed intake and weight gain were affected negatively by 7.5 and 7.1%, respectively, from 1 to 42 d of age, while FCR was not significantly affected in broiler chickens reared under a hot and humid tropical climate. These results suggested that broilers were more sensitive to feeding the reduced-CP diet during the starter phase compared to the finisher. This apparent lesser sensitivity of older heat-stressed broilers to feeding reduced-CP diets compared to their counterpart heat-stressed birds at an early age could be explained by the higher feed consumption of older broilers, which allows the birds to compensate during later ages.

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Conclusions

The response of broilers to reduced-CP diets under heat stress conditions is affected by a number of factors. The severity of heat exposure plays a major role in determining the beneficial or detrimental effects of feeding reduced-CP diets on broiler chickens reared at high environmental temperatures. The reduced-CP diets seem to be beneficial when broilers are subjected to an average daily ambient temperature of ≤27.3°C. However, feeding reduced-CP diets seem to be detrimental to broilers reared under chronic heat stress conditions. Only a narrow margin of CP may be reduced through EAA supplementation, while dietary supplementation with Gly may allow for greater CP reduction without compromising the growth performance of broilers exposed to moderate heat stress. Despite the fact that older broilers are more susceptible to heat

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stress conditions, younger heat-stressed broilers seem to be more sensitive to CP reductions.

References

AFTAB, U., ASHRAF, M. and JIANG, Z. (2006) Low protein diets for broilers. *World's Poultry Science Journal* **62**: 688-701.

AKINDE, D.O. (2014a) Amino acid efficiency with dietary Gly supplementation: Part 1. *World's Poultry Science Journal* **70**: 461-474.

AKINDE, D.O. (2014b) Amino acid efficiency with dietary Gly supplementation: Part 2. *World's Poultry Science Journal* **70**: 575-584.

ALLEMAN, F. and LECLERCQ, B. (1997) Effect of dietary protein and environmental temperature on growth performance and water consumption of male broiler chickens. *British Poultry Science* **38**: 607-610.

ARBOR ACRES (2014) Arbor acres plus broiler nutrition specifications. *Aviagen Huntsville, Alabama, USA*.

AUSTIC, R. (1985) Stress physiology in livestock, Journal, Vol. 3, (Boca Raton, FL, CRC Press).

AWAD, E.A., FADLULLAH, M., ZULKIFLI, I., SOLEIMANI, A.F. and LOH, T.C. (2014a) Amino acids fortification of low-protein diet for broilers under tropical climate: Ideal essential amino acids profile. *Italian Journal of Animal Science* **13**: 270-274.

AWAD, E.A., IDRUS, Z., SOLEIMANI FARJAM, A., BELLO, A.U. and JAHROMI, M.F. (2018) Growth performance, duodenal morphology and the caecal microbial population in female broiler chickens fed glycine-fortified low protein diets under heat stress conditions. *British Poultry Science* **59**: 340-348.

AWAD, E.A., ZULKIFLI, I., SOLEIMANI, A.F. and ALJUOBORI, A. (2017) Effects of feeding male and female broiler chickens on low-protein diets fortified with different dietary glycine levels under the hot and humid tropical climate. *Italian Journal of Animal Science* **16**: 453-461.

AWAD, E.A., ZULKIFLI, I., SOLEIMANI, A.F. and LOH, T.C. (2014b) Amino acids fortification of low-protein diet for broilers under tropical climate. 2. Nonessential amino acids and increasing essential amino acids. *Italian Journal of Animal Science* **13**: 631-636.

AWAD, E.A., ZULKIFLI, I., SOLEIMANI, A.F. and LOH, T.C. (2015) Individual non-essential amino acids fortification of a low-protein diet for broilers under the hot and humid tropical climate. *Poultry Science* **94**: 2772-2777.

BAKER, D.H. (1997) Ideal amino acid profiles for swine and poultry and their applications in feed formulation, Journal, Vol. 9, pp. 15-19 (Cape Girardeau, MO, Biokyowa).

BALNAVE, D. and BARKE, J. (2002) Re-evaluation of the classical dietary arginine: Lysine interaction for modern poultry diets: A review. *World's Poultry Science Journal* **58**: 275-289.

BALNAVE, D., HAYAT, J. and BRAKE, J. (1999) Dietary arginine: Lysine ratio and methionine activity at elevated environmental temperatures. *The Journal of Applied Poultry Research* **8**: 1-9.

BALNAVE, D. and OLIVA, A. (1990) Responses of finishing broilers at high temperatures to dietary methionine source and supplementation levels. *Crop and Pasture Science* **41**: 557-564.

CAHANER, A., PINCHASOV, Y., NIR, I. and NITSAN, Z. (1995) Effects of dietary protein under high ambient temperature on body weight, breast meat yield, and abdominal fat deposition of broiler stocks differing in growth rate and fatness. *Poultry Science* **74**: 968-975.

CHAMRUSPOLLERT, M., PESTI, G. and BAKALLI, R. (2004) Influence of temperature on the arginine and methionine requirements of young broiler chicks. *The Journal of Applied Poultry Research* **13**: 628-638.

CHENG, T.K., HAMRE, M.L. and COON, C.N. (1997) Effect of environmental temperature, dietary protein, and energy levels on broiler performance. *The Journal of Applied Poultry Research* **6**: 1-17.

CHENG, T.K., HAMRE, M.L. and COON, C.N. (1999) Effect of constant and cyclic environmental temperatures, dietary protein, and amino acid levels on broiler performance. *The Journal of Applied Poultry Research* **8**: 426-439.

COBB (2015) Cobb 500 broiler performance and nutrition supplement. Cobb-Vantress, Siloam Springs, Arkansas, USA.

CORZO, A., MORAN, E. and HOEHLER, D. (2003) Lysine needs of summer-reared male broilers from six to eight weeks of age. *Poultry Science* **82**: 1602-1607.

D'MELLO, J.P.F. (2003) Responses of growing poultry to amino acids, in: D'MELLO, J.P.F. (Ed) *Amino Acids in Animal Nutrition, 2nd Edition*, pp. 237-263 (Wallinford, UK, CAB International).

DAGHIR, N.J. (2008) Poultry production in hot climates (Wallinford, UK, CAB International).

DOZIER, W., CORZO, A., KIDD, M. and SCHILLING, M. (2008) Dietary digestible lysine requirements of male and female broilers from forty-nine to sixty-three days of age. *Poultry Science* **87**: 1385-1391.

DOZIER, W., CORZO, A., KIDD, M., TILLMAN, P. and BRANTON, S. (2009) Digestible lysine requirements of male and female broilers from fourteen to twenty-eight days of age. *Poultry Science* **88**: 1676-1682.

https://doi.org/10.1017/S0043933919000576 Published online by Cambridge University Press

DOZIER, W., MORAN, E. and KIDD, M. (2001) Comparisons of male and female broiler responses to dietary threonine from 42 to 56 days of age. *The Journal of Applied Poultry Research* **10**: 53-59.

FARIA FILHO, D., ROSA, P., VIEIRA, B., MACARI, M. and FURLAN, R.L. (2005) Protein levels and environmental temperature effects on carcass characteristics, performance, and nitrogen excretion of broiler chickens from 7 to 21 days of age. *Revista Brasileira de Ciência Avícola* **7**: 247-253.

FARIA FILHO, D.D., CAMPOS, D.M.B., ALFONSO-TORRES, K.A., VIEIRA, B.S., ROSA, P.S., VAZ, A.M., MACARI, M. and FURLAN, R.L. (2007) Protein levels for heat-exposed broilers: Performance, nutrients digestibility, and energy and protein metabolism. *International Journal of Poultry Science* **6**: 187-194.

GONZALEZ-ESQUERRA, R. and LEESON, S. (2005) Effects of acute *vs.* chronic heat stress on broiler response to dietary protein. *Poultry Science* **84**: 1562-1569.

HAN, Y. and BAKER, D.H. (1993) Effects of sex, heat stress, body weight, and genetic strain on the dietary lysine requirement of broiler chicks. *Poultry Science* **72**: 701-708.

HAN, Y. and BAKER, D.H. (1994) Digestible lysine requirement of male and female broiler chicks during the period three to six weeks posthatching. *Poultry Science* **73**: 1739-1745.

HUBBARD (2014) Broiler management guide. *Hubbard S.A.S., Quintin, France.*

HUNCHAR, J.G. and THOMAS, O. (1976) The tryptophan requirement of male and female broilers during the 4-7 week period. *Poultry Science* **55**: 379-383.

KESSLER, J.W. and THOMAS, O. (1976) The arginine requirement of the 4-7 week old broiler. *Poultry Science* **55**: 2379-2382.

LARA, L.J. and ROSTAGNO, M.H. (2013) Impact of heat stress on poultry production. *Animals* **3**: 356-369.

LAUDADIO, V., DAMBROSIO, A., NORMANNO, G., KHAN, R.U., NAZ, S., ROWGHANI, E. and TUFARELLI, V. (2012) Effect of reducing dietary protein level on performance responses and some microbiological aspects of broiler chickens under summer environmental conditions. *Avian Biology Research* **5**: 88-92.

LAW, F.L., ZULKIFLI, I., SOLEIMANI, A.F., LIANG, J.B. and AWAD, E.A. (2018) The effects of low-protein diets and protease supplementation on broiler chickens in a hot and humid tropical environment. *Asian-Australasian Journal of Animal Sciences* **31**: 1291-1300.

LAW, F.L., ZULKIFLI, I., SOLEIMANI, A.F., LIANG, J.B. and AWAD, E.A. (2019) Effects of protease supplementation of low protein and/or energy diets on growth performance and blood parameters in broiler chickens under heat stress condition. *Italian Journal of Animal Science* **18**: 679-689.

LIU, Q.W., FENG, J.H., CHAO, Z., CHEN, Y., WEI, L.M., WANG, F., SUN, R.P. and ZHANG, M.H. (2016) The influences of ambient temperature and crude protein levels on performance and serum biochemical parameters in broilers. *Journal of Animal Physiology and Animal Nutrition* **100**: 301-308.

MENDES, A., WATKINS, S., ENGLAND, J., SALEH, E., WALDROUP, A. and WALDROUP, P. (1997) Influence of dietary lysine levels and arginine: Lysine ratios on performance of broilers exposed to heat or cold stress during the period of three to six weeks of age. *Poultry Science* **76**: 472-481.

MORAN, E. (2010) Absorptive surface, mucin and phytin. Proceedings of the 1st International Phytase Summit, pp. 91-99.

MUSHARAF, N.A. and LATSHAW, J.D. (1999) Heat increment as affected by protein and amino acid nutrition. *World's Poultry Science Journal* **55**: 233-240.

NRC (1994) Nutrient requirements of poultry. 9th revised edition (Washington, DC, National Academy Press).

OJANO-DIRAIN, C. and WALDROUP, P. (2002) Evaluation of lysine, methionine and threonine needs of broilers three to six week of age under moderate temperature stress. *International Journal of Poultry Science* **1**: 16-21.

ROSS (2014) Ross 308 broiler: Nutrition specifications. *Aviagen Huntsville, Alabama, USA.*

SCOTT, M.L., NESHEIM, M.C. and YOUNG, R.J. (1982) Nutrition of the chicken (Ithaca, NY, Scott and Associates, Publishers).

SHAN, A., STERLING, K., PESTI, G., BAKALLI, R., DRIVER, J. and ATENCIO, T. (2002) The influence of temperature on the threonine requirement of young broiler chicks. Proceedings of the Poultry Science Association 91st Ann Meet Abstracts, August, pp. 11-14.

SINURAT, A. and BALNAVE, D. (1985) Effect of dietary amino acids and metabolisable energy on the performance of broilers kept at high temperatures. *British Poultry Science* **26**: 117-128.

TEETER, R. (1994) Optimizing production of heat stressed broilers. *Poultry Digest* **53**: 10-27.

TEMIM, S., CHAGNEAU, A.M., GUILLAUMIN, S., MICHEL, J., PERESSON, R., GERAERT, P.A. and TESSERAUD, S. (1999) Effects of chronic heat exposure and protein intake on growth performance, nitrogen retention and muscle development in broiler chickens. *Reproduction Nutrition Development* **39**: 145-156.

TEMIM, S., CHAGNEAU, A.M., GUILLAUMIN, S., MICHEL, J., PERESSON, R. and TESSERAUD, S. (2000) Does excess dietary protein improve growth performance and carcass characteristics in heat-exposed chickens? *Poultry Science* **79**: 312-317.

https://doi.org/10.1017/S0043933919000576 Published online by Cambridge University Press

THOMAS, O., FARRAN, M., TAMPLIN, C. and ZUCKERMAN, A. (1987) Broiler starter studies. I. The threonine requirements of male and female broiler chicks. Ii. The body composition of males fed varying levels of protein and energy. Proceedings-Maryland Nutrition Conference for Feed Manufacturers (USA).

THOMAS, O., ZUCKERMAN, A., FARRAN, M. and TAMPLIN, C. (1986) Updated amino acid requirements of broilers. Proceedings-Maryland Nutrition Conference for Feed Manufacturers (USA).

WALDROUP, P.W. (1982) Influence of environmental temperature on protein and amino acid needs of poultry. Proceedings of the Federation Proceedings, pp. 2821-2823.

ZAMAN, Q.U., MUSHTAQ, T., NAWAZ, H., MIRZA, M.A., MAHMOOD, S., AHMAD, T., BABAR, M. E. and MUSHTAQ, M.M.H. (2008) Effect of varying dietary energy and protein on broiler performance in hot climate. *Animal Feed Science and Technology* **146**: 302-312.

ZULKIFLI, I., AKMAL, A.F., SOLEIMANI, A.F., HOSSAIN, M.A. and AWAD, E.A. (2018) Effects of low-protein diets on acute phase proteins and heat shock protein 70 responses, and growth performance in broiler chickens under heat stress condition. *Poultry Science* **97**: 1306-1314.

https://doi.org/10.1017/S0043933919000576 Published online by Cambridge University Press

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Table 3 Latest recommended crude protein and amino acids specifications for as-hatched Arbor Acres, Cobb500, Hubbard, and Ross308 broilers[1]

	Starter		Grower		Finisher 1		Finisher 2		Finisher 3	
Age period for Arbor Acres and Ross308 (d)	0-10		11-24		25-39		40-46		>46	
Age period for Cobb500 (d)	0-10		11-22		23-42		>42			
Age period for Hubbard (d)[2]	0-10		11-20		21-33		34-42		>42	
Crude protein (%)										
Arbor Acres and Ross308	23		21.5		19.5		18.0		17.5	
Cobb500	21-22		19-20		18-19		17-18		-	
Hubbard	22-24		22-24		20-22		19-21		17-19	
Amino acids (%)	Total	Digest[3]	Total	Digest[3]	Total	Digest[3]	Total	Digest[3]	Total	Digest[3]
Lysine										
Arbor Acres and Ross308	1.44	1.28	1.29	1.15	1.15	1.02	1.08	0.96	1.04	0.93
Cobb500	1.32	1.18	1.19	1.05	1.05	0.95	1.00	0.90		
Hubbard	1.40	1.23	1.40	1.23	1.25	1.06	1.15	0.98	0.95	0.81
Methionine + Cysteine										
Arbor Acres and Ross308	1.08	0.95	0.99	0.87	0.90	0.80	0.85	0.75	0.82	0.73
Cobb500	0.98	0.88	0.89	0.80	0.82	0.74	0.78	0.70		
Hubbard	1.05	0.90	1.05	0.90	0.98	0.85	0.90	0.78	0.78	0.68
Threonine										
Arbor Acres and Ross308	0.97	0.86	0.88	0.77	0.78	0.68	0.73	0.64	0.71	0.62
Cobb500	0.86	0.78	0.78	0.69	0.71	0.65	0.68	0.61		
Hubbard	0.90	0.78	0.90	0.78	0.85	0.72	0.78	0.67	0.67	0.57
Valine										
Arbor Acres and Ross308	1.10	0.96	1.00	0.87	0.89	0.78	0.86	0.75	0.83	0.73
Cobb500	1.00	0.89	0.91	0.81	0.81	0.73	0.77	0.69		
Hubbard	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Isoleucine										
Arbor Acres and Ross308	0.97	0.86	0.89	0.78	0.80	0.70	0.75	0.66	0.73	0.64
Cobb500	NS	NS	NS	NS	NS	NS	NS	NS		
Hubbard	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Arginine										
Arbor Acres and Ross308	1.52	1.37	1.37	1.23	1.21	1.09	1.15	1.04	1.12	1.00
Cobb500	1.38	1.24	1.25	1.1	1.13	1.03	1.08	0.97		
Hubbard	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

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Table 3 Continued

	Starter		Grower		Finisher 1		Finisher 2		Finisher 3	
Tryptophan										
Arbor Acres and Ross308	0.23	0.20	0.21	0.18	0.18	0.16	0.17	0.15	0.17	0.15
Cobb500	0.20	0.18	0.19	0.17	0.19	0.17	0.18	0.16		
Hubbard	0.24	0.22	0.24	0.22	0.22	0.19	0.21	0.18	0.16	0.14
Leucine										
Arbor Acres and Ross308	1.58	1.41	1.42	1.27	1.26	1.12	1.19	1.06	1.15	1`.02
Cobb500	NS	NS	NS	NS	NS	NS	NS	NS		
Hubbard	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1]Amino acids specifications in this table were obtained from the latest broiler nutrition specifications guides of Arbor Acres (2014), Cobb (2015), Hubbard (2014) and Ross (2014).
[2]The age periods 0-10, 11-20, 21-33, 34-42, and >42 d are considered as pre-starter, starter, grower, finisher, and withdrawal, respectively.
[3]Digest = digestible
NS = not specified

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Table 4 Effects of reduced-protein diets on growth performance in broilers reared under various heat stress conditions.

Heat stress condition	Average daily temperature (°C)	Dietary CP level (%)[1]		Age (day)	Supplemental amino acids[3]	Growth performance response[4]			Reference
		Control diet[2]	Reduced-CP diet			FI/ADFI	BW/WG/ ADG	FCR	
Constant HS (32°C)	32.0	19.9	16.0	23-44	Met, Lys,Thr, Arg and Val	ns	-16.0%	-9.9%	Alleman and Leclercq (1997)
Constant HS (32.2°C)	32.2	20.0	16.0	21-49	Met and Lys	ns	-26.2%	-17.1%	Cheng *et al.* (1999)
Cyclic HS (35°C for 8 h)	29.4	20.0	16.0	21-49	Met and Lys	-21.7%	-28.9%	ns	Cheng *et al.* (1999)
Constant HS (33°C)	33.0	21.5	18.5	7-21	All EAA except Phe, Leu and His	ns	-6.0%	-5.7%	Faria Filho *et al.* (2005)
Constant HS (27.3°C)	27.3	20.0	18.0	21-42	Met, Lys,Thr and Arg	ns	Ns	ns	Gonzalez-Esquerra and Leeson (2005)
Constant HS (31.4°C)	31.4	20.0	18.0	21-42	Met, Lys,Thr and Arg	ns	Ns	-6.2%	Gonzalez-Esquerra and Leeson (2005)
Constant HS (32.2°C)	32.2	20.0	17.0	21-42	All EAA except Phe, Leu and His	ns	Ns	-9.0%	Faria Filho *et al.* (2007)
Hot climate	NA[7]	20.8	18.8	1-28	Met, Lys and Thr	ns	Ns	ns	Zaman *et al.* (2008)
Summer conditions	NA[7]	20.5	18.5	14-42	Met, Lys and Thr	ns	Ns	ns	Laudadio *et al.* (2012)
Tropical climate[5]	At least 28.3	20.6	19.2	1-21	All EAA except Phe, Leu and His	ns	Ns	ns	Awad *et al.* (2014a)
Tropical climate[5]	At least 28.3	20.6	17.9	1-21	All EAA except Phe and Leu	ns	-10%	-8.8%	Awad *et al.* (2014a)
Tropical climate[5]	At least 28.1	22.3	16.8	1-21	All EAA + Gly	-5.6%	-8.6%	-4.4%	Awad *et al.* (2015)
Cyclic HS[6]	27.8	18.2	14.9	28-42	Met, Lys, Thr and Trp	ns	Ns	-8.5%	Liu *et al.* (2016)
Tropical climate[5]	At least 28.1	22.3	17.3	1-21	All EAA + Gly	-7.8	-10.9%	-3.6%	Awad *et al.* (2017)
Tropical climate[5]	At least 28.3	22.3 and 19.4	17.3 and 14.5	1-21/ 22-42	All EAA + Gly	-7.5%	-7.1%	ns	Awad *et al.* (2017)
Cyclic HS (33°C for 6 h)	25.5%	19.1	16.2	22-35	Met, Lys,Thr and Trp	ns	-33.6%	-13.1%	Zulkifli *et al.* (2018)
Tropical climate[5]	At least 28.5	21.0 and 18.5	19.0 and 16.7	1-21/ 22-35	Met, Lys,Thr and Trp	ns	-9.1%	-8.1%	Law *et al.* (2018)

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Table 4 Continued

Heat stress condition	Average daily temperature (°C)	Dietary CP level (%)[1]		Age (day)	Supplemental amino acids[3]	Growth performance response[4]			Reference
Constant HS (34°C)	34.0	19.4	14.3	22-42	All EAA + Gly	-28.3%	-40.1%	-16.6%	Awad *et al.* (2018)
Cyclic HS (34°C for 7 h)	26.2	19.4	14.3	22-42	All EAA + Gly	ns	Ns	ns	(Awad *et al.* (2018))
Cyclic HS (34°C for 7 h)	26.2	19.0	16.7	22-42	Met, Lys,Thr and Trp	ns	Ns	ns	(Law *et al.* (2019))

HS, heat stress; CP, crude protein; EAA, essential amino acids; FI, feed intake; ADFI, average daily feed intake; BW, body weight; WG, weight gain; ADG, average daily gain; FCR, feed conversion ratio.

[1] Values represent analysed dietary crude protein or calculated crude protein if the analysed values are not presented.

[2] When the study investigated various CP levels with some higher than the recommended CP level for the particular age, a diet with CP level equal/close to recommended CP level was considered as the control diet.

[3] Diets were fortified with the mentioned EAA to meet the requirements.

[4] ''ns'' indicates non-significant difference between the control and reduced-CP diets; ''+'' sign indicates a significant positive response to reduced-CP diet compared to control diet;

''-''sign indicates a negative response to reduced-CP diet compared to control diet.

[5] A natural (uncontrolled) hot and humid environment with maximum (at least 6 h per day) and minimum temperatures of 36°C and 26°C, respectively.

[6] 32°C for 4 h , 28°C for 16 h to 23°C for 4 h.

[7] NA indicates the difficulty to estimate the average daily temperature from the details of the environmental condition provided by authors.