Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2019 / v.21 / n.3 / 001-006

http://dx.doi.org/10.1590/1806-9061-2018-0962

Original Article

Techniques to Minimize the Effects of Acute Heat Stress or Chronic in Broilers

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■Keywords

Animal nutrition, broilers, electrolyte balance, pale muscle syndrome, temperature.

Submitted: 06/December/2018
Approved: 23/April/2019

ABSTRACT

High environmental temperature is limiting factor in broiler production. In order to minimize the undesirable consequences of acute or chronic heat stress, the techniques of fixed dietary electrolyte balance and early heat conditioning were evaluated. The objective of this study was to evaluate the possible interactions and effects of dietary electrolyte balance and early heat conditioning on feed intake, body weight, feed conversion ratio, mortality, energy bioeconomic index, fecal moisture, abdominal fat, and breast meat color (L*a*b*) of broilers submitted to chronic or acute heat stress. In total, 1280 chicks, were equally divided in experiment I (chronic heat stress, 6h/day at 32°C from 35 to 39 d of age) and II (acute heat stress, 36 °C for 6h at 38 days of age). The data of both experiments were combined and analyzed according to a 2x2x2 factorial arrangement (early heat conditioning (ETC) or not; fixed dietary electrolyte balance (EB) or not; and exposure to acute or chronic heat stress). ETC consisted of exposing 5-d-old birds to 36.0 °C for 24 hours. No interaction among the evaluated factors was detected. Birds exposed to acute heat stress presented significantly higher compared with chronic heat stress. Fixed dietary EB resulted in significantly higher fecal moisture. Lower abdominal fat percentage was obtained in birds exposed to chronic relative to acute heat stress. Higher breast meat L* and b* values were observed in birds exposed to acute heat stress than those submitted to chronic heat stress, indicating worse meat quality.

INTRODUCTION

One of the main challenges in commercial broiler production is heat stress and its economic consequences, as it reduces growth rate and feed intake, and increases mortality (Mujahid, 2011). The expansion of the poultry industry to tropical regions has stimulated the search for methods to alleviate the consequences of heat stress (Zaboli *et al.*, 2017). Although Brazil is the world's second largest chicken meat producer, with 13.05 million tons (ABPA, 2018) and has a promising market, its tropical climate, with high temperatures and high relative humidity, poses a continuous challenge to live broiler performance.

The expansion of the poultry industry to tropical regions has stimulated the search for methods to alleviate the consequences of heat stress (Zaboli *et al.*, 2017). The acclimation of birds, dietary and/or drinking water management, the use of antipyretic drugs and ascorbic acid, as well as the manipulation of dietary protein, energy and major electrolyte levels have been employed (Borges *et al.*, 2003).

Heat stress causes several metabolic changes in broilers, including acid-base imbalance. In an attempt to prevent such effects and to improve animal performance, Gamba *et al.* (2015) suggested that diets should be formulated with adequate electrolyte levels and ratios aiming at obtaining appropriate electrolyte balance. There are few literature reports on the potassium (K), chorine (Cl), and sodium (Na) ratio for broilers, expressed as $([K^+] + [Cl^-])/[Na^+]$ (Ahmad & Sarwar, 2006), although this concept had already been proposed in 1981 by Mongin (1981), possibly due to the complexity of its calculation in feed formulation. However, the Solver tool of the Microsoft® Excel spreadsheets allows introducing the mathematical equations required to calculate both electrolyte balance and ratio (ER) during feed formulation (Garcia Neto, 2008).

Broilers can also be physiologically manipulated to better tolerate heat stress using acclimation or heat conditioning. By exposing broilers to constant high environmental temperature, acclimation promote heat tolerance; however, it impairs live performance. On the other hand, thermal conditioning by short-term exposure of chicks to high temperatures during the first week of age results in thermotolerance and maintains the growth rate, but depends on compensatory weight gain (Yahav & McMurtry, 2001).

The objective of this study was to evaluate the possible interactions between early thermal conditioning (ETC) and diet formulation considering electrolyte balance (EB) on the performance, carcass traits, as well as their economic viability in broilers submitted to acute or chronic heat stress.

MATERIALS AND METHODS

The study was carried out in accordance with the Ethical Principles of Animal Experimentation (COBEA) and approved by Animal Ethics Committee, under process n. 00887-2012.

Two experiments were performed. Each evaluated 640 Cobb 500 male broilers from 1 to 42 days of age. In experiment I, birds were submitted to chronic heat stress, and to acute stress in experiment II; both were applied in the final third of the rearing period.

One-day-old broilers were weighed and randomly distributed in metal cages with 20 birds each. Each cage was equipped with an automated heating source (60W bulb). At 5 days of age, half of the flock of each experiment (320 birds from experiment I and 320 from experiment II) was submitted to the Early Heat conditioning (ETC), which consisted of exposing the birds to a temperature of 36 °C for 24 hours in their original cages. The other half was placed in the rearing shed.

On d 6, all birds were placed in an environmentally-controlled masonry shed, with an adiabatic evaporative cooling system, negative pressure ventilation, and wood-shavings litter. Water and feed were provided *ad libitum*.

A 3-phase feeding regime was applied (starter, grower, and finisher diets). The diets were formulated considering fixed electrolyte balances (EB) and ratios (ER) or not (conventional diets). Dietary electrolyte balance was calculated as $EB = Na^+ + K^+ - Cl^-$ and electrolyte ratio as $ER = (K^+ + Cl^-)/Na^+$, according to Mongin (1981). The following values were considered in the formulation: EB = 250 mEq/kg and ER = 3:1 in the starter diet, and EB = 300 mEq/kg and ER = 3:1 in the grower and finisher diets (Table 1).

The treatments consisted of the combination or not of dietary electrolyte balance (EB), early heat conditioning (ETC) and exposure to chronic (Exp. I) or acute (Exp. II) heat stress (Table 2).

In experiment I, birds were submitted to chronic heat stress, which consisted of exposure to an average temperature of 32°C for 6 h/d from 35 to 39 days of age. In experiment II, the birds were submitted to acute heat stress, which consisted of exposure to an average temperature of 36°C for 6 h at 38 days of age. These temperatures were obtained by switching off the cooling system and turning on the heating system (Gonzalez-Esquerra & Leeson, 2005).

As no interaction between treatments and experiments (p>0.05) was detected, the data from experiment I and II were combined according to the procedure adopted by Cromwell *et al.* (1995). A number of 1,280 broiler was distributed according to a completely randomized experimental design in 2x2x2 a factorial arrangement (ETC or not, diets with fixed EB or not, and exposure or not to chronic or acute heat stress), totaling eight treatments, with eight replicates each.

On day 42, corrected feed intake (kg/bird/period), corrected feed conversion ratio (kg of feed/kg body weight) and weight gain (kg/bird/period) were evaluated. Birds were observed twice daily (morning and afternoon), and all mortality was accounted for the correction of feed intake and feed conversion ratio.

The economic viability of the experimental diets was determined by the Bioeconomic Energy Conversion Index (BEC) (Garcia Neto *et al.*, 2013), according to the equation below. It is based on the total weight of the flock (kg live birds at the end of the production cycle) and not average weight, and, therefore, allows taking into account mortality (Figure 1).

Table1 – Composition and cost of the diets fed to the broiler during experiments I and II according to rearing stage.

	Unit	Calculated Composition					
		Conventional diet			Diet with fixed EB		
		Starter	Grower	Finisher	Starter	Grower	Finisher
Diet cost	BRL/100kg	76.81	74.53	67.15	78.53	78.37	71.49
Calculated nutritional composition							
Metabolizable energy	kcal/kg	2980	3050	3100	2980	3100	2980
Crude protein	%	20.62	19.14	17.84	20.66	19.13	17.83
Calcium	%	0.86	0.75	0.65	0.86	0.75	0.65
Available phosphorus	%	0.38	0.34	0.29	0.38	0.34	0.29
Potassium	%	0.81	0.75	0.69	0.85	0.98	0.97
Sodium	%	0.21	0.20	0.20	0.21	0.23	0.23
Chlorine	%	0.38	0.36	0.36	0.20	0.18	0.17
Linoleic acid	%	2.52	2.69	2.61	2.60	2.91	2.87
Digestible Lysine	%	1.14	1.05	0.97	1.14	1.05	0.97
Digestible Methionine	%	0.54	0.50	0.46	0.54	0.50	0.46
Digestible Methionine + Cystine	%	0.82	0.76	0.71	0.82	0.76	0.71
Digestible Threonine	%	0.74	0.68	0.63	0.74	0.68	0.63
Digestible Tryptophan	%	0.23	0.21	0.19	0.23	0.21	0.19
EB= K+Na-Cl (mEq/kg)*	mEq	191.70	176.27	160.94	250.00	300.00	300.00
ER=(K+Cl)/Na*	-	3.42	3.36	3.27	3.00	3.00	3.00
Ingredients							
Corn (7.88%)	%	59.32	63.44	68.02	58.79	62.07	66.45
Soybean meal (45%)	%	34.68	30.74	26.98	34.86	30.97	27.24
Soybean oil	%	2.06	2.30	2.04	2.22	2.77	2.58
Dicalcium phosphate	%	1.47	1.24	1.03	1.47	1.24	1.03
Calcitic limestone	%	1.06	0.94	0.83	1.06	0.94	0.83
Vitamin and mineral premix**	%	0.60	0.60	0.30	0.60	0.60	0.30
Sodium bicarbonate	%	0.00	0.00	0.00	0.41	0.55	0.57
Salt	%	0.48	0.46	0.44	0.20	0.16	0.13
L-Lysine	%	0.18	0.18	0.20	0.18	0.18	0.19
DL-Methionine	%	0.11	0.08	0.14	0.11	0.09	0.14
Potassium carbonate (99.5%)	%	0.00	0.00	0.00	0.07	0.42	0.51
L-Threonine	%	0.04	0.03	0.02	0.04	0.03	0.02

*EB= electrolyte balance; ER= electrolyte ratio

**Composition per kg of product: Starter diets: Vit. A – 1670,000 IU; Vit. E – 2.500 mg; Vit. K3 – 417 mg; Vit. B1 – 250 mg; Vit. B2 – 835 mg; Vit. B6 – 250 mg; Vit. B12 – 2,000 mg; Folic acid – 100 mg; Biotin – 9 mg; Niacin – ,835 mg; Calcium pantothenate – 1,870 mg; Cu – 1,000 mg; Co – 17 mg; I – 170 mg; Fe – 8,335 mg; Mn – 10,835 mg; Zn – 7,500 mg; Se – 35 mg; Choline chloride 50% - 116,670 mg; Methionine – 250,000 mg; Coccidiostat – 13,335 mg; Growth promoter – 13.335 mg; Antioxidant – 2000 mg. Grower diet: Vit. A –1,335,000 IU; Vit. D3 – 300,000 IU; Vit. E –2.000 mg; Vit. K3 –335 mg; Vit. B1 – 167 mg; Vit. B2 – 670 mg; Vit. B6 – 170 mg; Vit. B12 – 1,670 mg; Folic acid – 67 mg; Biotin – 7 mg; Niacin – 4.670 mg; calcium pantothenate – 1.870 mg; Cu – 1,000 mg; Co – 17 mg; I – 170 mg; Fe – 8,335 mg; Mn – 10,835 mg; Zn – 7,500 mg; Se – 35 mg; Choline chloride 50% - 83,340 mg; Methionine – 235,000 mg; Coccidiostat – 10,000 mg; Growth promoter – 10,000 mg; Antioxidant – 2,000 mg. Finisher diet: Vit. A – 1670,000 IU; Vit. D3 – 335.000 mg; Vit. E – 2,335 mg Vit. K3 – 400 mg; Vit. B1 – 100 mg; Vit. B2 – 800 mg; Vit. B6 – 200 mg; Vit. B12 – 2,000 mg; Folic acid – 67 mg; Biotin – 7 mg; Niacin – 5,670 mg; Calcium pantothenate – 2,000 mg; Cu – 2,000 mg; Co – 27 mg; I – 270 mg; Fe – 16,670 mg; Mn – 17,335 mg; Zn – 12,000 mg; Se – 35 mg; Choline chloride 50% - 100,000 mg; Methionine – 235,000 mg; Antioxidant – 2,000 mg.

Table 2 – Treatments evaluated in experiments I (chronic heat stress) and II (acute heat stress).

Treatments	Heat stress [1]	Fixed dietary electrolyte balance[2]	Early thermal conditioning[3]
A	Chronic (I)	No	No
B		Yes	Yes
C		No	Yes
D		Yes	No
E	Acute (II)	No	No
F		Yes	Yes
G		No	Yes
H		Yes	No

[1] Chronic heat stress – 32°C for 6 h/day between 35 and 39 days of age; Acute heat stress – 36°C for 6 h at 38 days of age

[2]Fixed dietary electrolyte balance (mEq/kg): EB= $(Na^+/22.99 + K^+/39.10 - Cl^-/35.45)*1000$; ER= $[(K^+/39.10 + Cl^-/35.45)/(Na^+/22.99)]$

[3]Early heat conditioning: at 5 days of age, birds were exposed to 36°C for 24 hours.

$$BEC = \frac{\left[(a1.b1.c1)+(a2.b2.c2)+(a3.b3.c3)\right]}{d.e} Mcal/kg$$

Figure 1 – Improved BEC index equation

Where:

a = feed intake (kg),

b = diet cost (R$/kg),

c = dietary metabolizable energy content (Mcal/kg),

d = final flock weight (kg),

e = price paid per kg of live chicken (R$/kg),

1 = starter phase

2 = grower phase

3 = finisher phase

Three birds per replicate (96 per experiment), which BW was representative of the average BW of the replicate, were stunned and sacrificed by bleeding. Carcasses were scalded, plucked, chilled, and eviscerated for determine fat and carcass weight.

The breasts were collected from the carcasses, deboned, the skin removed. Skinless and deboned breast samples (~200 g) were submitted to color analysis at 4 °C (Woelfel *et al.*, 2002), using a portable color spectrophotometer (Mini-Scan XE Plus, HunterLab, USA), calibrated with white and black standards. Breast color was determined according to the CIE L*a*b* color system (Comission Internationale de l'Eclairage), where "L" is luminosity (0 = black and 100 = white), "a" is the red/green coordinate (+a = red and -a = green) and "b" is the yellow/blue coordinate (+b = yellow and -b = blue).

On day 42, 32 fresh excreta samples (~10 g) were collected per replicate for the evaluation of fecal moisture. Excreta samples were weighed, pre-dried in a forced-ventilation oven at 55 °C for 72 h, dried in forced-ventilation oven at 105 °C for 12 hours, and then weighed in analytical precision Shimadzu scale (model bl-3200h, Japan), according to Silva & Queiroz (2002).

Data were submitted to analysis of variance to verify the effect of treatments and to evaluate the effects of each factor, according to PROC GLM procedures of the SAS statistical package (SAS Institute, 2000). Means were compared by Student's t-test was applied, and significance was established at 5% probability level.

RESULTS AND DISCUSSION

The results of experiments I and II were combined because no significant interactions (p>0.05) between

Table 3 – Effects of the combination of experiments I and II (application of chronic and acute heat stress, respectively) on average feed intake (IF), weight gain (WG), feed conversion ratio (FCR), fecal moisture (FM), mortality, bioeconomic energy conversion index (BEC), abdominal fat weight (AFW), and breast meat color characteristics (L* a* b*) of 42-d-old male broilers.

Factors	FI (kg)	WG (kg)	FCR	FM (%)	Mortality (%)	BEC[2] (Mcal/kg)	AFW (g)	Breast color[3]		
								L*	a*	b*
	Pr>F									
Fixed electrolyte balance (EB)										
Yes	5.71	3.42	1.81	81.70 a	34.81	3.11	91.48	62.40	8.18	20.60
No	5.70	3.34	1.81	80.19 b	33.88	2.73	89.91	61.97	8.25	20.21
Early thermal conditioning (ETC)										
Yes	5.74	3.37	1.82	80.66	36.06	2.98	92.23	62.36	8.19	20.62
No	5.66	3.39	1.81	81.22	32.63	2.84	89.16	61.99	8.24	20.20
Heat stress										
Chronic	5.70	3.40	1.81	80.85	3.06 a	1.74 a	53.90 a	57.66 a	8,06	17,64 a
Acute	5.70	3.35	1.81	81.01	65.63 b	4.20 b	127.49 b	66.40 b	8,37	23,17 b
CV (%)	9.26	7.22	8.77	3.17	37.31	44.13	13.91	3.86	11.74	5.61
ETC	0.5443	0.7676	0.7415	0.3488	0.2879	0.6922	0.3327	0.2513	0.8391	0.1510
EB	0.9397	0.2286	0.9495	0.0202	0.7709	0.1970	0.6189	0.4692	0.7891	0.1777
Thermal stress	0.9998	0.4160	0.9983	0.8826	<0.0001	<0.0001	<0.0001	<0.0001	0.2064	<0.0001
ETC * EB	0.4931	0.6173	0.6592	0.0563	0.4734	0.2589	0.8509	0.5116	0.5074	0.3101
ETC *heat stress	0.9308	0.9400	0.6059	0.6429	0.2879	0.7850	0.6756	0.0955	0.3855	0.1389
EB * heat stress	0.7597	0.6718	0.9957	0.5500	0.4975	0.2832	0.4793	0.1179	0.5425	0.6909
ETC * EB * heat stress	0.8384	0.2993	0.9683	0.7485	0.4051	0.2255	0.5381	0.2666	0.7660	0.1250

[1]Means followed by the different letters in the same column within each factor are different by the Tukey's test at 5% probability level.

[2]BEC (bioenergy conversion cost) = [(feed intake (kg) x dietary metabolizable energy content (Mcal/kg) x diet cost (R$/kg))/(final flock weight (kg) x price paid per kg of live chicken (R$))]

[3]L*= lightness – express as a percentage (from 0 for black to 100 for white); a*= red/green coordinate: + a= red, - a= green; b*= yellow/blue coordinate: + b= yellow, - b= blue; Available at: htt0://sensing.konicaminolta.com.br/2013/11/entendendo-oespaco-de-cor-lab/#sthash.5fVQkPAX.dpuf

treatments and experiments were detected, i.e., the effects of the evaluated treatments were independent of the experiments (I and II). In addition, there was no interaction among or between the treatments. The treatments did not influence (p>0.05) feed intake, weight gain, or feed conversion ratio (Table 3).

The diets with fixed EB and the early heat conditioning were not effective in minimizing the effects of neither acute or chronic heat stress, as previously found by Oliveira *et al.* (2016) in a study with broilers subjected to chronic heat stress.

Fecal moisture was influenced only by dietary electrolyte balance (p=0.0202), possibly due to the higher dietary levels of the ions Na^+ e K^+ (Table 1). Excessive Na^+ increases water consumption, whereas excessive K^+ results in increased urine output (Araújo *et al.*, 2010), which may result in higher litter moisture. Borges *et al.* (2003) showed that the addition of potassium chlorine (KCl) to the drinking water or diet promotes water consumption in broilers reared under heat stress. The addition of KCl is frequently recommended to increase water consumption of broilers under heat stress to support body heat loss mechanisms (Smith & Teeter, 1987; Borges *et al.*, 2003; Souza *et al.*, 2002). However, as water consumption increases, there is a proportional increase in fecal moisture, leading to poor litter quality, and consequently, impairing broiler performance and making flock management more difficult (Gamba *et al.*, 2015). Therefore, increased fecal moisture is related to excessive Na^+ and K^+ intake as well as to heat stress. When environmental temperatures are higher than upper thermal comfort limit (18 to 20 °C), water consumption can reach 0.5 liters/bird/day. According to Teeter *et al.* (1985), higher water intake may be beneficial to broilers under heat stress conditions; however, in the present experiment, no such benefit was found in birds submitted to chronic heat stress.

The main reasons of the high dietary potassium content were possibly: 1) the main protein source of protein included on the experimental diets was soybean meal, which contains high potassium level (Araújo *et al.*, 2010); and 2) and the inclusion of potassium carbonate in the experimental diets with fixed electrolyte balance (EB = 300 mEq/kg and ER = 3). However, this did not occur in the starter feed, because K requirement was fixed in EB = 250 mEq/kg. Another possibility would be to use a different protein source with lower potassium concentration.

No interactions (p>0.05) among the evaluated factors were detected for mortality, and there was no effect of ETC or diet on this parameter. However, mortality was 21.44 times higher (p<0.001) in broilers submitted to acute heat stress (65.63%) compared with those challenged with chronic stress (3.06%). The high temperature (36°C) to which birds were subjected during acute stress possibly caused heat shock, leading to death (Ludtke *et al.*, 2010). During periods of heat stress, the body undergoes several metabolic and physiological changes as the birds attempt to stay alive; however, these changes are limited, and may cause death when overcome (Borges *et al.*, 2003).

No interactions (p>0.05) among the evaluated factors were detected for the bioeconomic index, and there was no effect (p>0.05) of ETC or diet on this parameter. On the other hand, worse BEI (p<0.001) was calculated for the broilers submitted to acute heat stress (4.20) compared with those reared under chronic heat stress (1.74). This result is attributed to the higher mortality due to acute heat stress, as final body weight of the flock is considered in the calculation of the BEC index. These findings are in agreement with those reported by Deaton *et al.* (1986), Plavnik & Yahav (1998), and Tabler *et al.* (2002), who showed that acute heat stress causes high mortality, and, consequently, high losses in the investments made in the production process of broilers and costs associated with the correct disposal of the dead birds.

No interactions (p>0.05) among the evaluated factors were detected for abdominal fat deposition (g), and there was no effect (p>0.05) of ETC or diet on this parameter. However, birds reared under chronic heat stress (127.5g) presented 2.37 times lower (p<0,0001) abdominal fat weight than those submitted to acute heat stress (53.9g). This result may be explained by the utilization of body energy reserves (body fat) during the heat stress period. The behavioral responses of broilers to heat stress include erection of feather follicles and spreading wings of the body, increased consumption of cold water, panting, and drastically reduced feed intake (Teeter *et al.*, 1985, Vale *et al.*, 2010). Therefore, the longer the period of exposure to heat stress, the greater the utilization of energy reserves. In a study comparing chronic and acute heat stress in broilers, Xie *et al.* (2015) found that acute stress results in blood metabolism disorders, whereas chronic stress causes tissue damage due to the greater period of exposure the high temperatures.

Breast meat color was not affected (p>0.05) by the interactions among factors, nor by ETC and dietary fixed EB balance. The results show, however, lower L* and b* (p<0.0001) values in the breast meat of birds submitted to acute heat stress. In addition to the high mortality, acute heat stress resulted in pale breast meat, with mean L* value of 66.22, consistent with the L* value previously found by Smith & Northcutt (2009) in the breast meat of heat-shocked broilers (L * = 68.4). Heat stress immediately prior to slaughter negatively affects the color of turkey meat (Ngoka & Froning, 1982) and broilers (Northcutt *et al.*, 1994). The present experiment confirmed that acute heat stress may impair chicken meat color. In addition, meat water holding capacity decreases as L* values increase, resulting in lower tenderness (Ludtke *et al.*, 2010). Therefore, chicken breast meat with high L* values are less tender and paler, which are undesirable from the consumer's perspective.

The results of this study show that feeding diets with fixed electrolyte balance (EB) and early thermal conditioning (ETC) were not effective in alleviating the negative effects caused by chronic and acute heat stress in broiler chickens. However, acute heat stress causes higher economic losses and poorer carcass quality compared with chronic heat stress in broilers.

ACKNOWLEDGMENTS

The authors thank the financial support granted by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) under process number 13/09537-7.

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