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Effects of the Addition of Pure Glycerin Supplementation in the Drinking Water on the Performance of Broilers Submitted to Heat Stress and Feed Restriction

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ABSTRACT

The experiment was conducted to study the effect of pure glycerin supplementation (GLYC) in the drinking water of broilers subjected to heat stress and feed restriction. Water with 0, 1, or 2% glycerin was provided *ad libitum* to broilers in six hours of feed restriction. The birds were housed in two environments: thermoneutral (TN) - 25 ° C, and cyclic heat stress (HS) - 12h with 25^{0}C, 6h with 32^{0}C, 3h of 25^0 to 32^{0}C and 3h of 32^0 to 25^{0}C. The experimental design was randomized in a factorial arrangement of 2 x 3 + 2, with three GLYC levels, two environments and one control group with *ad libitum* feeding each environment. When submitted to HS, broiler receiving 2% glycerin presented higher weight gain (WG), water consumption (WC), feed intake (FI) and energy consumption (EI) than those in the other treatments, contrarily to birds in TN, where increasing GLYC levels decreased those responses. Broilers submitted to feed restriction presented reduced FI, but better feed conversion (FCR), independently of rearing environment or GLYC levels. Broilers under HS submitted to feed restriction and receiving 2% GLYC presented higher WC (p<0.05) and similar WG as the controls, differently from the restricted-fed broilers under TN receiving 2% GLYC, which WC and WG were lower than the controls. The inclusion of 2% pure glycerin in the drinking water may compensate the negative effects on performance caused by feed restriction in broilers submitted to heat stress.

INTRODUCTION

Studies on glycerol consumption via drinking water for animals and human athletes have shown that this substance has hyper-moisturizing properties due to the osmotic effect of glycerol in the body, supporting body fluid regulation when physical activities are performed in high environmental temperatures (Schott *et al.*, 2001; Coutts *et al.*, 2002; Kavouras *et al.*, 2006; Patlar *et al.*, 2012).

The survival of poultry under heat stress largely depends on water consumption, because this nutrient is associated with thermoregulatory mechanisms. The capacity of an animal to regulate its body temperature depends on the balance between heat production and heat dissipation (Yalçin *et al.*, 1997). In high temperature environments, chickens decrease their feed intake to reduce the heat increment produced from feed metabolism, resulting in worse performance (Macari *et al.*, 2002; Ribeiro *et al.*, 2008; Oba *et al.*, 2012).

An alternative method to help to control body temperature restriction feed offer before and during the hottest hours of the day. Reducing feed intake decreased the metabolic rate, and therefore, body temperature. Several studies showed that the body temperature and the mortality rate of broilers under heat stress submitted to feed restriction are reduced (Yahav*et al.*, 1996; De Basilio *et al.*, 2001; Yalçin *et al.*, 1997). However, the reduced feed intake resulting from feed restriction leads to low weight gain (Özkan *et al.*, 2003; Barbour *et al.*, 2010). In addition, water consumption during the period of feed removal may also decrease, reducing the efficiency of heat loss (Barbour *et al.*, 2010).

Energy sources, such as glycerin, may compensate for feed restriction and, at the same time, stimulate water consumption. Although there are many studies showed positive results of the glycerin as an energy supplement for athlete animals and humans on hot days (Schott *et al.*, 2001; Kavouras *et al.*, 2006; Dozier *et al.*, 2008; Patlar *et al.*, 2012; Romano *et al.*, 2014), there are no studies on the effects of glycerin inclusion in the drinking water of broilers submitted to heat stress. Therefore, the objective of this study was to evaluate the supplementation of pure glycerin the drinking water on the performance of broilers submitted to feed restriction and to cyclic heat stress.

MATERIAL AND METHODS

Pure GLYC (99% glycerol) levels of 0, 1, or 2% were supplied to broilers kept under heat stress and total feed restriction during hottest part of the day (for six hours) for a period of seven days. Glycerin was daily mixed with the drinking water in 20-L containers, and kept cool until the mixture was placed in the drinkers.

Two hundred and forty 35-d-old male Cobb broilers, with2158 ± 69 g body initial weight, were housed in cages, equipped with tube feeders and trough drinkers, divided into two rooms: TN (thermoneutral environment) and HS (cyclic heat stress). Cyclic HS consisted of 12 hours at 25 °C, three hours of temperature increase from 25 to 32 °C, six hours at 32 °C, and three hours of temperature decrease from 32 to 25 °C daily, as shown in Figure 1. In the TN room, daily temperatures ranged between 21 and 25 °C. Relative humidity was maintained at approximately 70% in both environments (Figure 1).

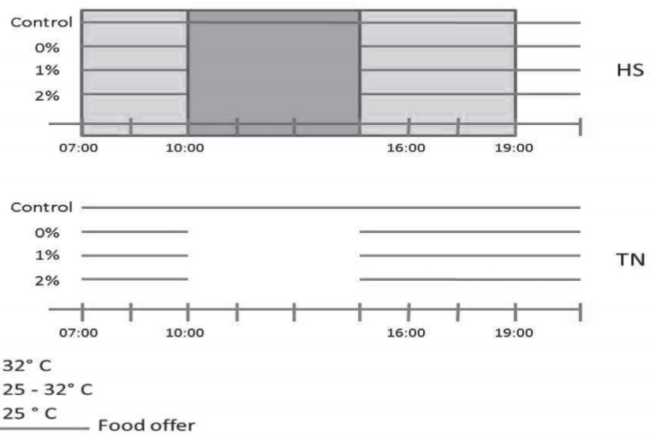

Figure 1 – Description of the daily temperature distribution in the thermoneutral (TN) and heat stress (HS) environments, and of the feed restriction period.

All birds were fed a diet based on corn and soybean meal (Table 1), formulated to meet the broilers'nutritional requirements (Rostagno *et al.*, 2011). In each room, a control treatment, in which birds were fed *ad libitum*, was maintained

Table1 – Ingredients and calculated nutritional composition of the diets fed to broilers between 35-42 days of age.

Ingredient	Composition (%)
Corn	63.48
Soybean meal (45%)	29.15
Soy oil	4.25
Dicalcium phosphate	1.11
Limestone	0.84
Salt	0.43
L-Lysine HCl	0.24
DL-Methionine	0.26
L-Threonine	0.05
Mineral / vitamin premix[1]	0.12
Anticoccidial agent[2]	0.02
Choline chloride 60%	0.04
Nutritional Composition	
AMEn (kcal kg^{-1})	3200.00
CP (%)	18.59
Calcium (%)	0.66
Available phosphorus	0.31
Potassium (%)	0.72
Sodium (%)	0.19
Chlorine (%)	0.31
Lysine (%)	1.06
Methionine + Cystine (%)	0.77

[1]Composition (content per kgpremix): 150,000 mg Mn, 100,000 mg Zn, 80,000 mg Fe, 15,000 mg Cu, 1,200 mg I, 700 mg Se, 23,200,000 IU vitamin A, 5,600,000 IU vitamin D, 52,000 mg vitamin K, 6,000 mgvitamin B1, 18,000 mg vitamin B2, 9,000 mg vitamin B6, 13,2000 mgniacin, 44,000 mg pantothenic acid, 2,400 mgfolic acid, 200,000 µgbiotin, 40,000 µg vitamin B12.

[2]Monensin - 100 ppm.

AMEn: apparent metabolizable energy corrected for nitrogen; CP: crude protein

Feed intake (FI) and water consumption (WC) were daily measured in two periods: during feed restriction (6 h) and during the subsequent period (18h). Weight gain (WG), feed conversion ratio (FCR), and mortality were determined at the end of the experimental period. Total energy intake (EI) was calculated as the sum of the feed and water energy intake. Daily feed energy intake was calculated as dietary metabolizable energy level multiplied by feed intake. Water energy intake was estimated as pure glycerin metabolizable energy level (3400 kcal/kg, according to Gianfelici, 2009) multiplied by water consumption.

Birds were distributed according to a completely randomized experimental design in a 2 x 3 + 2 arrangement, consisting of two environments (TN and HS) and three glycerol supplementation levels (0%, 1% and 2%), plus one control group (receiving feed *ad* *libitum*) in each environment, totaling eight treatments with six replicates of five birds each.

Data were analyzed using PROC GLM (SAS 9.2). The factorial arrangement was analyzed by analysis of variance, and included the effects of glycerin supplementation levels, environment, and their interactions for all variables studied. The control (feed *ad libitum*) and feed restriction treatments were compared by three orthogonal contrasts for each environment: control *vs.* feed restriction with 0% glycerin, control *vs.* feed restriction with 1% glycerin, control *vs.* feed restriction with 2% glycerin.

RESULTS AND DISCUSSION

The analysis of the 2 x 3 factorial arrangement showed an interaction of GLYC levels with the environment for the parameters FI, WC, WG, and EI during the restriction and subsequent period. Therefore, the main effects are not discussed, but the effects of GLYC supplementation levels in each environment.

In the HS environment, FI and WC were not different when birds consumed 0and 1% GLYC; however, birds supplemented with 2% GLYC consumed more feed and water during both periods ($p<0.05$) than the first two groups. On the other hand, in the TN environment, 0% GLYC supplementation promoted the highest FI and the best FCR, and these parameters linearly worsened as supplementation levels increased (Figure 2, 3, 4 and 5). Under HS, the birds receiving2% GLYC presented higher WG ($p<0.05$) compared with those fed 0and 1% GLYC, which WG was similar ($p>0.05$), opposite to the WG results obtained in the TN environment, where 2% GLYC promoted the worst WG results (Figure 6). The worse performance observed with increasing GLYC levels in the broilers maintained in the TN environment was unique and not consistent with literature findings on the inclusion of GLYC as an energy source in the diet. Crude GLYC (±86% glycerol) can be included up to 50g/kg diet with no adverse effects on broiler performance (Cerrate *et al.*, 2006; Guerra *et al.*, 2011). The inclusion of high glycerol levels exceeds the metabolization capacity of the enzyme glycerol kinase, resulting in excessive glycerol excretion (Cerrate *et al.*, 2006; Guerra *et al.*, 2011). During the finisher phase, that inclusion level corresponded to 8.6 g glycerol daily intake, which is close to the 9g consumed by the birds fed 2% GLYC and kept under TN, therefore not characterized as excessive.

Under heat stress, high water consumption is beneficial, because water allows for evaporative heat loss in broilers (Smith & Teeter, 1992). The latter observed that the water intake and weight gain of

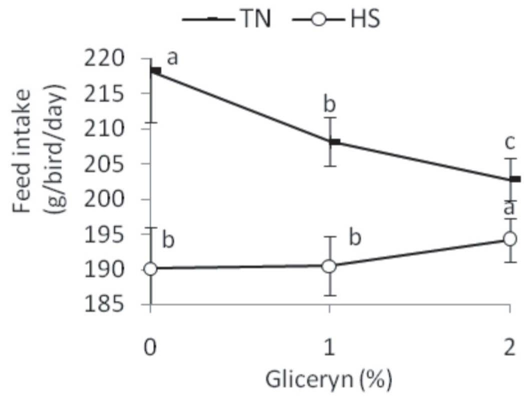

Figure 2 – Feed intake in TN and HS. Means ± SE followed by different letters in the same row differ.

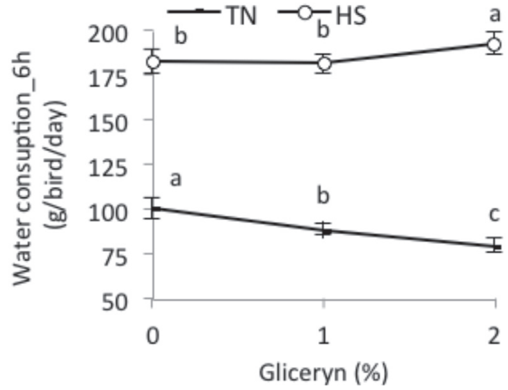

Figure 3 – Water consumption during the fasting period in TN and HS. Means ± SE followed by different letters in the same row differ.

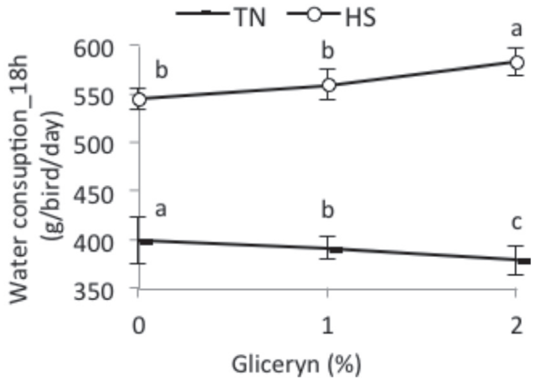

Figure 4 – Water consumption in 18h of broilers in TN and HS. Means ± SE followed by different letters in the same row differ.

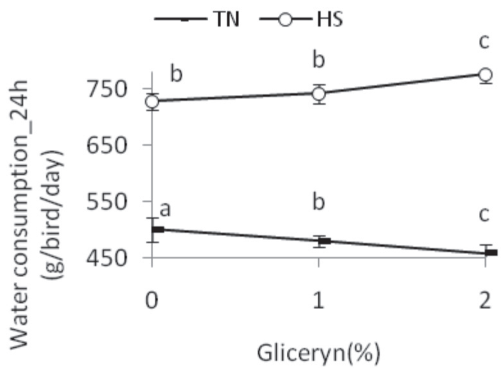

Figure 5 – Water consumption during 24h in broilers at ATN and HS. Means ± SE followed by different letters in the same row differ.

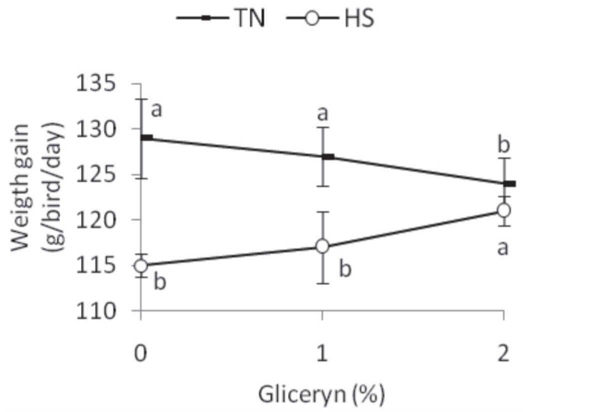

Figure 6 – Weight gain in TN and HS. Means ± SE followed by different letters in the same row differ.

broilers under cyclic heat stress and supplemented with 0.2% potassium chloride via drinking water significantly increased, suggesting that higher weight gains are related with higher water consumption. According to Mushtaq *et al.* (2013), electrolyte intake, as it changes plasma osmolarity, induces thirst, causing the animal to drink more water. Similar to electrolytes, glycerol also changes plasma osmolality. Coutts *et al.* (2002) conducted a study with triathlete men consuming beverages with 1.2 g glycerol/kg body weight and observed, when tests were performed on hot days, performance improvement, a reduction in urine output, and increased retention of body fluids compared with individuals in the placebo group. Likewise, Schott *et al.* (2001) administered glycerol in saline solution by nasogastricroute to horses and observed an increase in water intake and hence, a hyper-moisturizing effect. This induction of hyper-hydration in high-temperature environments results in greater fluid retention compared with ingestion of pure water, and it is related to the rapid absorption and osmotic activity of glycerol (O'Brien *et al.*, 2005).

In addition to its osmotic and moisturizing effect, increasing circulating levels of glycerol stimulate hepatic gluconeogenesis, increasing the number of substrates available for energy supply (Kavouras *et al.*, 2006). Under HS, EI was increased with increasing GLYC levels ($p<0.05$), because the supplementation with 2% GLYC contributed with 53 kcal, in addition of the energy consumed in the feed. This may also have contributed to the higher WG observed in broilers receiving 2% GLYC and submitted to HS (Figure 6). Moreover, in the TN environment, 1 and 2% GLYC in the drinking water reduced EI, suggesting that the addition of GLYC in the drinking water supplied part of the broilers' energy requirements, and therefore, they needed to obtain less energy from the diet (Figure 7), whereas in broilers maintained in HS presented lower feed intake.

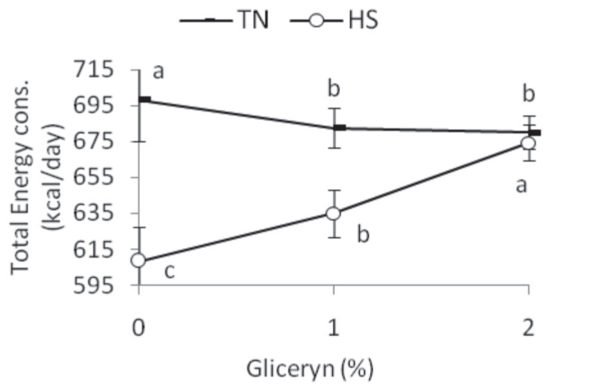

Figure 7 – Total broiler energy intake (feed +water) in TN and HS. Means ± SE followed by different letters in the same row differ.

Under HS, the control group presented the worst FCR ($p<0.05$), whereas the birds receiving 2% GLYC presented similar WG ($p>0.05$) and higher WC ($p<0.05$) than the controls, indicating that this level of GLYC supplementation in the drinking water may alleviate the adverse effects caused by feed restriction on broiler performance (Table 2). Broilers receiving 0% and 1% GLYC presented lower WG compared with the controls, but similar WC. In the TN environment, independently of GLYC level, the birds who suffered feed restriction consumed less feed and less water than the controls, but presented better FCR (Table 2),

Table 2 – Performance of broilers fed *ad libitum* (control) or submitted to feed restriction and supplemented with 0, 1, or 2 % glycerin and reared in thermoneutral or heat-stress environments.

Heat stress				
	FI_24h	WC_24h	WG	FCR
Control	219	730	118	1.86
Feed restriction (0% Glyc)	190	727	115	1.66
Feed restriction (1% Glyc)	190	741	116	1.64
Feed restriction (2% Glyc)	194	776	120	1.62
CV (%)	2.2	2.8	2.3	2.9
CONTRAST 1	*	ns	*	*
CONTRAST 2	*	ns	*	*
CONTRAST 3	*	*	ns	*
Thermoneutral environment				
Treatment	FI_24h	WC_24h	WG	FCR
Control	233	544	129	1.80
Feed restriction (0% Glyc)	209	499	129	1.62
Feed restriction (1% Glyc)	208	480	129	1.60
Feed restriction (2% Glyc)	202	458	124	1.62
CV (%)	2.6	4.1	3.2	2.4
CONTRAST 1	*	*	ns	*
CONTRAST 2	*	*	ns	*
CONTRAST 3	*	*	*	*

FI_24h: feed intake during a 24h period; WC_24h: Water consumptionduring a 24h period; WG: Weight gain; BW: Body weight; FCR: feed conversion ratio; CV: coefficient of variation. ns: no significant; * significant at $p<0.05$.
Contrast 1 – Control *vs.* 0% glycerin
Contrast 2 – Control *vs.* 1% glycerin
Contrast 3 – Control *vs.* 2% glycerin

in agreement with the findings of Butzen *et al.* (2013) and Furlan *et al.* (2002). The WG of the birds receiving 0%and 1% GLYC was not different relative to the controls, but those fed 2% GLYC presented the lowest WG ($p<0.05$), which may be explained by their lower WC and FI compared with those receiving 0% and 1% GLYC.

Feed restriction is a management practice recommended to prevent the mortality of broilers reared under high temperature and humidity (Ribeiro & Laganá, 2002); however, no mortality was recorded in the present experiment (data not shown), possibly because the birds were reared at low density.

CONCLUSION

The inclusion of 2% pure glycerin in the drinking water may be used as a tool to improve the performance of broilers submitted to feed restriction and cyclic heat stress.

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