The Journal of Animal & Plant Sciences, 29(1): 2019, Page: 33-40
ISSN: 1018-7081

PHYSIOLOGICAL PARAMETERS AND THERMAL COMFORT INDICES OF LAYERS FED VEGETABLE GLYCERIN

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ABSTRACT

The aim of this study was to evaluate the physiological parameters and thermal comfort indices of commercial layers fed diets containing different levels of vegetable glycerin. A total of 378 Hy-Line White layers at 32 weeks of age were housed in a shed at nine birds per cage. The experiment lasted 84 days. Treatments consisted of a control diet (without glycerin) plus five diets with increasing levels of vegetable glycerin (3, 6, 9, 12, and 15%). The black globe humidity index (BGHI) and radiant heat load (RHL) were out of the thermal comfort zone and were higher in the afternoon period in all production cycles. Respiratory frequency was lower in the morning, when 3% glycerin were added to the diet. The surface temperatures of comb, wattle, back, wings, head, and feet did not differ with the glycerin levels and were higher in the 2nd cycle, but within the range recommended for the species. Glycerin inclusion levels of up to 12% can be used to partially replace corn in diets for layers housed in sheds covered with ceramic tiles without compromising their thermoregulation in a hot environment.

Keywords: Alternative Feeds. Homeothermy. Poultry Farming. Thermal Environment.

INTRODUCTION

Poultry farming is an activity of great importance in the Brazilian agribusiness sector, and its products are indispensable in the diet of the majority of the population. It is one of the segments of greatest prominence today, mainly in view of its national expansion. This situation has placed Brazil in an outstanding position in the world scenario thanks to not only the produced volume but also the quality of products commercialized.

With an increasingly demanding market in terms of products developed in compliance with minimal animal-welfare standards, greater attention has been paid to the comfort of animals in their rearing facilities aiming at their welfare and consequent higher production potential. In the architectural planning process, it is important to take into account the climatic reality of each region, considering mainly the natural thermal conditioning. When exposed to heat stress in the poultry house, animals have a decline in production, which is a physiological response in an effort return to the thermal comfort zone (Damasceno *et al*. 2010) whereby more energy is expended to maintain the body within the thermoneutral zone.

The thermoneutral zone for layer hens to express their production potential in the thermal environment is when the temperature is between 21 and 28 ºC (Castilho *et al*., 2015) and the air relative humidity is between 50 and 70% (Tinôco, 2001). These can be used together with other environmental variables to calculate the thermal comfort indices.

An animal exposed to heat stress, especially inside facilities, has production losses due to the physiological response generated to return to the thermal comfort zone (Damasceno *et al*., 2010), expending more energy in this process. When under heat stress, animals like layers and broilers decrease their feed intake; for this reason, energy feedstuffs are sought to meet their energy requirements without compromising homeothermy in hot environment.

Because it has a similar metabolic energy to corn (Lammers *et al*., 2008), vegetable glycerin has gained relevance in animal nutrition. Studies on the use of glycerin as an energy source replacing corn in diets for Japanese quail have not demonstrated negative effects on the productive or reproductive performance of those birds (Ghayas *et al.,* 2017). In its crude state, it is a product with approximately 3,200 kcal of metabolizable energy for pigs and 3,600 kcal for broilers and layers (Menten *et al*, 2008).

A great deal of research is conducted with glycerin included at different levels in poultry diets. However, the ideal percentage to be used and its effects on performance and egg quality are yet to be defined (Cufadar *et al.,* 2016). In the metabolism of broilers, for instance, diets with high levels of glycerin may lead to metabolic alterations such as increases in blood glycerol, water intake, and fecal moisture (Romano *et al*., 2014).

According to Fontinelle *et al*. (2017), glycerin can partially replace corn at up to 10% without compromising the production performance or egg quality of brown-egg layers.

In an attempt to obtain deeper information on thermoregulation and glycerin levels in poultry diets, this study proposes to evaluate the physiological responses and thermal comfort indices of commercial layers fed diets containing different levels of vegetable glycerin in Sobral - CE, Brazil.

MATERIALS AND METHODS

Experimental Location: The experiment was conducted in the Poultry Unit at the Experimental Farm of the Department of Animal Science, Center for Agricultural and Biological Sciences, State University of Vale do Acaraú - UVA, located in Sobral - CE, Brazil

Animals and Experimental Design: A total of 378 commercial layers of the Hy-line White strain at 32 weeks of age, weighing 1.450 ± 0.077 kg, were used in a completely randomized experimental design with six treatments and seven replicates per treatment. Birds were weighed individually before the experiment and housed in 42 galvanized-wire cages containing three partitions per 30 × 45 cm cage for three birds, totaling nine birds per cage. Cages were located inside a galvanized-flat-wire closed shed measuring 12 m in length by 8 m in width, with a ceiling height of 2.60 m, covered with ceramic tiles. Curtains were located externally only on the west side. The experimental period was from October 2015 to January 2016, consisting of three 28-day cycles.

Diets Experimental: Diets were iso-nutrient and isoenergetic (Table 1) and formulated according to the manual of the Brazilian Hy-line strain (2015). Nutrients were supplemented according to the tables of nutritional requirements of poultry and swine, following Rostagno *et al.* (2011).

Table 1. Centesimal and calculated nutritional composition of the experimental diet.

Ingredient	Vegetable glycerin (%)					
	0	3	6	9	12	15
Grain corn	59.3056	55.4769	51.6483	47.8206	43.9954	40.1700
Soybean meal (45%)	21.2904	22.0145	22.7385	23.4611	24.1806	24.9001
Limestone	8.2568	8.2436	8.2304	8.2172	8.2040	8.1908
Glycerin	0	3.000	6.000	9.000	12.000	15.000
Meat meal	7.2920	7.3192	7.3464	7.3737	7.4010	7.4283
PX LAYER *	0.4000	0.4000	0.4000	0.4000	0.4000	0.4000
Common salt	0.2925	0.2935	0.2946	0.2956	0.2966	0.2977
Soybean oil	2.8321	2.9343	3.0366	3.1383	3.2388	3.3393
DL-methionine	0.1663	0.1678	0.1692	0.1716	0.1758	0.1800
L-lysine	0.1643	0.1502	0.1360	0.1219	0.1078	0.0938
	Calculated nutritional composition					
Metabolizable energy (kcal/kg)	2,900	2,900	2,900	2,900	2,900	2,900
Crude Protein (%)	18.000	18.000	18.000	18.000	18.000	18.000
Calcium (%)	4.3000	4.3000	4.3000	4.3000	4.3000	4.3000
Available phosphorus (%)	0.5400	0.5400	0.5400	0.5400	0.5400	0.5400
Sodium (%)	0.2000	0.2000	0.2000	0.2000	0.2000	0.2000
Potassium (%)	0.6126	0.6149	0.6173	0.6196	0.6219	0.6242
Chloride (%)	0.2499	0.2488	0.2477	0.2467	0.2456	0.2446
Dig. methionine + cystine (%)	0.7071	0.7045	0.7018	0.7000	0.7000	0.7000
Dig. methionine (%)	0.4600	0.4600	0.4600	0.4608	0.4635	0.4662
Dig. lysine (%)	0.9000	0.9000	0.9000	0.9000	0.9000	0.9000
Dig. isoleucine (%)	0.6206	0.6247	0.6289	0.6330	0.6371	0.6412
Dig. threonine (%)	0.5617	0.5628	0.5639	0.5650	0.5661	0.5671
Dig. tryptophan (%)	0.1674	0.1697	0.1720	0.1743	0.1766	0.1789
Dig. valine (%)	0.7022	0.7030	0.7038	0.7046	0.7053	0.7061

*7422 - PX LAYER 0.4% 500 TEC - Provides per kg: iron (min) - 10.00 g/kg; copper (min) - 2,500.00 mg/kg; zinc (min) - 20.00 g/kg; manganese (min) - 20.00 g/kg; iodine (min) - 208.00 mg/kg; selenium (min) - 75.15 mg/kg; vit. A (min) - 2,000,000.00 IU/kg; vit. D3 (min) - 625,000.00 IU/kg; vit. E (min) - 3,000.00; vit. K3 (min) - 395.92 mg/kg; folic acid (min) - 74.25 mg/kg; choline (min) - 100.00 g/kg; niacin (min) - 5,025.74 mg/kg; pantothenic acid (min) - 1,805.16 mg/kg; vit. B1 (min) - 250.09 mg/kg; vit. B2 (min) - 1,000.00 mg/kg; vit. B6 (min) - 250.10 mg/kg; vit. B12 (min) - 2,400.00 mcg/kg; methionine (min) - 125.00 g/kg; colistin (min) - 1,750.00 mg/kg.

The tested treatments were a control diet (without vegetable glycerin) and diets with increasing levels of glycerin (3, 6, 9, 12, and 15%) obtained from a refinery of Petrobrás S.A. in Quixadá - CE, Brazil (Table 2).

Table 2. Composition and characteristics of the glycerin used in the experiment (fresh-matter basis).

Item[1]	Value
Glycerol[1] %	76.5
Sodium chloride[1] %	5.3
Ash[1] %	5.3
Non-glycerin organic matter[1] %	0.67
Absolute density[1] kg/m^3	1,242. 3
pH[1]	5.5
Methanol[1] %	0.16
Moisture[1] %	17.6
Aspect[1]	Limpid
Color[1]	Yellow
Metabolizable energy for poultry	3,510 kcal/kg

[1-]Laboratory of the Petrobrás S.A. Refinery in Quixadá-CE, Braziol; [2-]Rostagno *et al.* (2011).

Data measurement: One bird was chosen at random per cage and its respiratory frequency was observed during 15 s; this value was when multiplied by four to obtain the number of movements per minute. Cloacal temperature (CT) was measured using a digital clinical thermometer that was inserted to a depth of three centimeters for 2 min. The surface temperatures of skin, comb, wattle, back, wing, feet, head, and cloaca were measured with an infrared digital thermometer (ºC) with no contact with the skin, at a distance of approximately 15 cm from the birds' body. All measurements were taken on three days per week, at 09h00 and 14h00, on the same day as the collection of environmental variables, during the entire experimental period.

Meteorological variables were recorded every 2 h, from 08h00 to 16h00, using a thermo-hygrometer (dry and wet bulb temperature), a maximum-minimum temperature thermometer, a black globe thermometer, and an anemometer (INMET, 2016). All the equipment was installed inside the shed, at a height of 1 m above the floor.

All birds received the same feeding and sanitary management during the evaluation period, with water available *ad libitum*. The feed was supplied in trough feeders placed in front of each experimental unit, while the water was supplied via nipple drinkers inside each partition of the cage.

Statistical analysis: Results were subjected to analysis of variance and means were compared by the SNK (Student-Newman-Keuls) test at the 5% significance level (SAS, 2000) and later analyzed in a factorial model including the effects of treatments (glycerin levels) periods, and the interaction between the factors.

RESULTS AND DISCUSSION

Between the 1st and 3rd production cycles, the highest average air temperatures (AT) were recorded in the afternoon period (Table 3). Both periods of the day were outside of the thermoneutral zone for layers, which is 15-28 ºC (Ferreira 2005), indicating that the birds inside the shed were possibly out of the thermal comfort zone. The wind speed (WS), whose highest value found in the 1st cycle was 0.3 m/s^2 in both periods (morning and afternoon), is within the range of 0.2 to 3.0 m/s^2 indicated by Ferreira (2005).

Table 3. Means plus standard deviations of Meteorological variables and thermal comfort indices recorded during the three production cycles of layer hens reared in Sobral - CE, Brazil.

	Variable					
Cycle	AT (ºC)	WS (m/s)	ARH (%)	BGT (ºC)	BGHI (ºC)	RHL (W/m^{-2})
			Morning			
1st	30.92±0.75	0.33±0.26	94.28±1.90	33.23±1.48	85.40±1.42	521.03±26.51
2nd	31.19±0.48	0.17±0.04	96.45±1.71	32.42±0.7	84.82±0.82	501.18±5.68
3rd	30.4±1.00	0.29±0.18	96.19±1.05	30.99±1.38	83.10±1.72	487.46±14.08
			Afternoon			
1st	35.19±1.01	0.33±0.26	94.53±3.71	35.8±1.25	89.49±1.78	522.22±13.48
2nd	35.47±0.95	0.17±0.04	96.60±1.73	36.39±0.81	90.34±1.15	525.87±5.65
3rd	33.7±1.60	0.29±0.18	96.48±1.30	34.65±1.58	87.96±2.14	516.10±9.84

AT = ambient temperature; WS = wind speed; ARH = air relative humidity; BGT = black globe temperature; BGHI = black globe humidity index; RHL = radiant heat load.

Mean values observed for air relative humidity (ARH) during the experiment were much higher than the critical limits of 50 to 70% (Tinôco, 2001) and 40 to 80% (Ferreira, 2005) established in the literature. This implies that the birds were likely in thermal discomfort because of the sudden change in air humidity during this experimental period, which was highest in the afternoon during the 2nd cycle, triggering physiological mechanisms for body heat exchange with the environment such as dissipation of latent heat through an increase in respiratory frequency.

Brito Santos *et al*. (2014) studied the bioclimatology of the coastal, *agreste*, and semi-arid regions of the state of Sergipe, Brazil, for broiler and layer farming, and found mean monthly air relative humidity (ARH) values between 83 and 94% in the semi-arid region, which is similar to our findings.

The black globe temperature (BGT) was higher in the afternoon period, when the highest mean value of 36.39 ºC was recorded in the 2nd cycle, along with an AT of 35.47 ºC inside the facility, which might have caused greater thermal stress to the birds in this cycle. The shed where the birds were housed was covered with ceramic files, but their thermal insulation against both the cold and the heat was not sufficient to maintain the internal temperature of the shed.

Results lower than those found here were reported by Passini *et al.* (2013). These researchers evaluated an environmental intervention in the roofing and artificial ventilation on the thermal comfort indices of broilers and found respective BGT of 281.8 and 28.60 ºC for roofs with and without reflexive paint and 28.17 and 28.61 ºC for the environments with and without artificial ventilation.

The black-globe humidity index (BGHI) and radiant heat load (RHL) were out of the thermal comfort range. The highest values for the respective variables were found in the afternoon periods in all production cycles, as follows: 89.49, 90.34, and 87.96 ºC; and 522.22, 525.87, and 516.10 (1st, 2nd, and 3rd cycles). In a study conducted by Biaggioni *et al.* (2008) evaluating the thermal performance of a layer hen farm conditioned naturally, the authors found BGHI of 79.09 ºC in the spring and 79.65 ºC in the summer during the afternoon period. In an experiment developed by Rosa (2009), the HRL was 515.4 W/m^{-2}.

Values similar to the above-mentioned ones were found by Jácome *et al*. (2007), who evaluated the thermal comfort indices of layer facilities in Northeast Brazil and recorded BGHI and HRL of 77.1 ºC and 469.4 W/m^{-2}, respectively, in sheds with ceramic-tile roofing for layers in the grower period.

The thermal comfort indices BGHI and HRL observed in this study might have been lower had we used roofing with reflexive paint and artificial ventilators as well as nebulizers in the sheds. The use of ceramic tiles did not benefit the internal environment of the shed to decrease the ambient temperature and provide the animals with a thermal comfort environment.

In a study on the welfare of layers at different housing densities, Castilho *et al*. (2015) found that rectal temperature reached 41.4 ºC at 16h00 with the housing densities of 10 and 12 birds per cage measuring 50×45×40 cm. In this study, the birds were housed in flat-wire cages with subdivisions measuring 35×40 at nine animals per cage, divided into three birds per partition, which might have compromised the heat exchange with the environment due to the small space per bird in each cage.

There was a difference (P<0.05) for the RF variable when the glycerin levels were increased to 15% in the diet, probably because glycerin is a feedstuff with a high energy content, which might have triggered an increase in panting to maintain the body temperature by maintaining metabolic heat loss though respiration. Additionally, in the three cycles, RF differed (P<0.05) in the afternoon, with a higher number of movements per minute (148.33 mov/min) recorded in the 1st cycle (Table 4).

The fact that RF showed a difference in the 1st cycle may also be related to the months of October and November, when the highest air temperatures in the studied region are recorded; nevertheless, the birds maintained homeothermy through an increase in panting. As for the vegetable glycerin, if added to the diet at levels lower than 15%, for instance, it can help the animal by supplying the energy expended to maintain homeothermy, thereby preventing a decrease in feed intake by the heat-stressed bird.

Garcia *et al.* (2015) analyzed the behavior of layers reared at different housing densities and observed that the respiratory frequency of this animal category can range from 23 mov/min, in a thermoneutral environment (20 ºC), to 273 mov/min, at high temperatures (35 ºC). This confirms the results found in the present study, where, at a higher temperature of 35.47 ºC, the average RF was 113.64 mov/min, recorded during the 2nd cycle.

Cloacal surface temperature and rectal temperature did not differ (P>0.05) and are within the reference range (Table 4). For all of the analyzed variables, the afternoon period presented the highest means when compared with the morning, since the highest ambient temperatures inside the poultry house were recorded in this period of the day. The highest cloacal ST, 37.05 ºC, was found during the 2nd cycle, which may be related to the higher BGT mean (Table 3) for the same cycle.

Castilho *et al*. (2015) examined the welfare of laying hens at different housing densities and reported that rectal temperature reached 41.4 ºC at 16h00 at 12 birds per cage and 41.3 ºC at 10 birds per cage, which is corroborated by the highest mean of 41.35 ºC found in our study in the 1st cycle at the density of nine birds per cage.

Different uppercase and lowercase letters in the same column differ statistically by the SNK test at the 5% probability level.

In this study, the birds were housed in flat-wire cages with 35 × 50-cm subdivisions containing nine animals per cage, divided into three per partition, which might have negatively interfered with the heat exchange with the environment because of the little space per bird inside the cages. Therefore, even in an environment with elevated temperatures, birds managed to maintain homeothermy. However, if RT were above the ideal 41ºC (Marchini *et al*., 2007), it would indicate that the mechanism of heat dissipation towards the thermal environment would not have been sufficient.

The surface temperatures of the birds as represented by the comb, wattle, back, wing, head, and feet points did not differ (P>0.05) with the dietary glycerin levels, but were higher in the afternoon period during the 2nd cycle (Table 5).

Comb and wattle temperatures were higher in the 2nd cycle. Similar values to those found in this study were reported by Sousa *et al*. (2016), who evaluated the comb and wattle temperatures as indictors of the thermoregulation of layer hens reared in the semi-arid region of Sobral - CE, Brazil, and recorded highest means for comb and wattle of 37.4 and 35.8 ºC, respectively, at 14h00. However, these values differed from those obtained at 09h00 by those authors.

In an environment above the thermal comfort zone, birds decrease their physical activity, consequently reducing their internal heat production. The blood then migrates to the comb and the wattle, where vasodilation occurs, causing these body parts to enlarge. In this way, the metabolic heat reaches the body extremities and is thus released to the environment by the processes of conduction, convection, and radiation (Melotti *et al*., 2011).

According to Nascimento and Silva (2010), the body surface temperature of birds is usually below the ambient temperature; in other words, if this temperature is higher than that of the environment, the animal is likely under thermal discomfort. Birds possess vasodilated extremities like the comb, the wattle, and the feet, and as the temperature increases, there is a greater flow of heat towards these areas, which are devoid of feathers, in an attempt for the animal to exchange heat with the environment and maintain homeothermy.

Camerini *et al*. (2016) investigated changes in the variation of surface temperature in layers grown in two rearing systems using thermography and found body, head, and feet temperatures of 29.44, 37.38, and 34.50 ºC, respectively, for birds reared in enriched cages at an ambient temperature of 32 ºC. The values for the same variables in the case of birds reared in alternative systems were 33.81, 38.70, and 38.91 ºC, respectively, at the same ambient temperature (32 ºC).

Table 4. Respiratory frequency (RF), cloacal surface temperature (ST), and rectal temperature (RT) of layer hens fed different levels of glycerin in Sobral - CE, Brazil.

Factor	1st cycle RF (mov/min)	Cloacal ST (ºC)	RT (ºC)
Glycerin level			
0%	122.75b	35.47	41.35
3%	57.50c	35.35	41.30
6%	112.00b	35.34	41.36
9%	120.86b	35.46	41.37
12%	101.50b	35.44	41.36
15%	149.75a	35.38	41.35
Period			
Morning	57.10 B	34.58B	41.04B
Afternoon	148.33 A	36.23A	41.67A
Mean	115.75	35.41	41.35
CV (%)	12.56	1.70	0.25
Analysis of variance			
Glycerin	0.0001	0.9873	0.5674
Period	0.0001	0.0001	0.0001
G × T	1.0000	0.7231	0.5300
		2nd cycle	
Glycerin level			
0%	118.55	37.30	41.37
3%	117.93	37.24	41.28
6%	115.48	37.13	41.34
9%	114.48	36.61	41.33
12%	109.43	36.96	41.30
15%	106.62	37.08	41.34
Period			
Morning	79.17 B	36.51B	41.05B
Afternoon	148.12 A	37.59A	41.60A
Mean	113.64	37.05	41.33
CV (%)	15.84	1.79	0.25
Analysis of variance			
Glycerin	0.4491	0.1054	0.3224
Period	0.0001	0.0001	0.0001
G × T	0.8271	0.9979	0.6366
		3rd cycle	
Glycerin level			
0%	99.07	36.22a	41.23
3%	102.82	35.63b	41.18
6%	97.21	36.15a	41.16
9%	97.68	35.92ab	41.20
12%	97.41	35.60b	41.16
15%	92.70	35.76ab	41.20
Period			
Morning	71.73 B	35.22B	40.98B
Afternoon	123.65 A	36.55A	41.39A
Mean	97.69	35.88	41.19
CV (%)	16.51	1.55	0.24
Analysis of variance			
Glycerin	0.7497	0.0155	0.5054
Period	<0.0001	<0.0001	<0.0001
G × P	0.8209	0.9691	0.9113

In this study, the birds were housed in flat-wire cages, and, in spite of the highest mean values of the 34.90 and 34.14 ºC and ambient temperature of 35 ºC in the 2nd cycle, the temperatures of head and feet were lower than those of birds reared in enriched cages and in alternative systems. Therefore, it can be inferred that the flat-wire cage as a rearing system did not negatively influence the temperatures of head and feet.

Table 5. Temperature (ºC) of the comb, wattle, back, wings, head, and feet of layer hens fed different levels of glycerin in Sobral - CE, Brazil.

			1sy cycle			
Factor	**Comb**	**Wattle**	**Back**	**Wings**	**Head**	**Feet**
Glycerin level						
0%	36.90	35.47	33.26ab	33.59	34.32	33.51
3%	36.98	35.35	33.37a	33.64	34.64	33.70
6%	36.95	35.34	33.47a	33.68	34.66	33.73
9%	36.92	35.46	32.96abc	33.35	34.35	33.51
12%	37.04	35.44	32.63bc	33.48	34.49	33.35
15%	36.94	35.38	32.68c	33.31	34.45	33.49
Period						
Morning	36.32B	34.58B	31.91B	32.33B	33.33B	32.46B
Afternoon	37.59A	36.23A	34.17A	34.68A	35.61A	34.63A
Mean	36.96	35.41	33.04	33.50	34.48	33.55
CV (%)	1.28	1.70	2.42	2.20	1.94	2.64
Analysis of variance						
Glycerin	0.9801	0.9873	0.0128	0.6901	0.7045	0.8753
Period	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
G × P	0.9066	0.7231	0.5164	0.9225	0.4729	0.9559
			2nd cycle			
Glycerin level						
0%	37.31	36.53	33.89ab	34.39	34.92	34.11
3%	37.20	36.74	33.98a	34.21	34.85	34.06
6%	37.34	36.53	34.11a	34.31	35.00	34.38
9%	37.14	36.52	33.80ab	34.23	34.94	34.12
12%	37.18	36.49	33.61ab	34.01	34.86	33.92
15%	37.18	36.64	33.09b	34.00	34.62	34.22
Period						
Morning	36.7 B	36.20B	32.69B	33.13B	33.89B	33.19B
Afternoon	37.74A	36.94A	34.79A	35.26A	35.92A	35.09A
Mean	37.22	36.57	33.74	34.19	34.90	34.14
CV (%)	0.93	1.61	2.40	1.91	1.60	2.20
Analysis of variance						
Glycerin	0.5917	0.8820	0.0227	0.5207	0.9649	0.7293
Period	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
G × P	0.6805	0.6784	0.7683	0.9780	0.8979	0.9804
			3rd cycle			
Glycerin level						
0%	36.59	33.89	32.70	33.01	34.24	32.98
3%	36.45	33.74	32.48	33.04	34.28	32.84
6%	36.59	33.62	32.81	33.09	34.34	33.07
9%	36.51	33.65	32.30	32.81	34.05	32.80
12%	36.60	33.72	32.45	32.98	34.09	32.70
15%	36.11	33.32	32.43	32.98	34.17	33.05
Period						
Morning	36.04B	33.01B	31.58B	32.00B	33.41B	32.17B
Afternoon	36.92A	34.30A	33.47A	33.94A	34.98A	33.64A
Mean	36.48	33.66	32.53	32.98	34.19	32.91
CV (%)	1.51	2.07	1.89	1.58	1.66	2.48
Analysis of variance						

Glycerin	0.1601	0.4090	0.2649	0.7939	0.7503	0.7904
Period	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
G × P	0.5457	0.8084	0.8374	0.9908	0.7316	0.9805

Different uppercase and lowercase letters in the same column differ statistically by the SNK test at the 5% probability level.

Conclusions: Glycerin inclusion levels of up to 12% can be used to partially replace corn in diets for layers housed in sheds with ceramic-tile roofing without compromising their thermoregulation in a hot environment.

Ethics statement: The project was approved by the Committee of Ethics in the Use of Animals (*Comissão de Ética no Uso de Animais* - CEUA) under case no. 004.07.015.UVA.504.03.

REFERENCES

Biaggioni, M.A.M., J.M. Mattos, S.P. Jasper, and L.A. Targa (2008). Thermal performance in layer hen house with natural acclimatization. Semina: Cienc. Agrar., Londrina, 29: 961-972.

Brito Santos, G., I.F. Sousa, C.O. Brito, V.S. Santos, R.J. Barbosa, and C. Soares (2014). Bioclimatic study for broiler production and posture in the of coastal, agreste and semi-arid regions of Sergipe State, Brazil. Cienc. Rural, Santa Maria, 44:123-128.

Camerini, N.L., R.C. Silva, J.W.B. Nascimento, D.L. Oliveira, and B.B. de Souza (2016). Surface temperature variation of laying hens created in two creation systems using thermography. Campina Grande. ACSA, 12:145-152.

Castilho, V.A.R., R.G. Garcia, N.D.S. Lima, K.C. Nunes, F.R. Caldara, I. A. Nääs, B. Barreto, and F.G. Jacob (2015). Welfare of laying hens in different densities of housing. UNESP, São Paulo. BIOENG, 9(2):122-131.

Cufadar, Y., R. Göçmen, and G. Kanbur (2016). The effect of replacing soya bean oil with glycerol in diets on performance, egg quality and egg fatty acid composition in laying hens. France. Animal, 10(1): 19-24.

Damasceno, F.A., L. Schiassi, J.A.O. Saraz, R.C.C. Gomes, and F.C. Baêta (2010). Architectural designs of plants used for poultry production in order to thermal comfort tropical and subtropical climates. Paraná. PUBVET, 4:986-991.

Ferreira, R.A. (2005). Greater production with a better environment for poultry, swine, and bovine. Aprenda Fácil, Viçosa (Brasil), 371p.

Fontinele, G.S.P., S.C. Bastos-Leite, C.N. Cordeiro, C.C. Goulart, A.C. Costa, J.O. Neves, and J.D.B. Silva (2017). Glycerin from biodiesel in the feeding of red-egg layers. Londrina. Semina: Ciênc Agrár. 38:1009-1016.

Garcia, E.R.M., K.C. Nunes, F.K. Cruz, A.L.J. Ferraz, N.R. Batista, and J.A. Barbosa Filho (2015). Behavior of laying hens raised in different population density accommodations. Umuarama. Arq. Ciênc. Vet. Zool. 18:87-93.

Ghayas, A., J. Hussain, A. Mahmud, K. Javed, A. Rehman, S. Ahmad, S. Mehmood, M. Usman, and H. M. Ishaq (2017). Productive performance, egg quality, and hatching traits of Japanese quail reared under different levels of glycerin. Poult. Sci. 96(7): 2226–2232.

Hy-Line do Brasil. (2015). Manual of the line: Commercial Hy-Line White Layers. Available at: <http://docplayer.com.br/15319883-Manual-de-manejo-w-36-poedeiras-comerciais.html>. Accessed August 01, 2015.

INMET. Meteorological Database for Teaching and Research - BDMEP. PDC. Available at: <http://www.inmet.gov.br/projetos/rede/pesquisa/>. Accessed February, 2016.

Jácome, I.M.T.D., D.A. Furtado, A.F. Leal, J.H.V. Silva, and J.F.P. Moura (2007). Evaluation of thermal comfort indexes for laying-hen houses in the northeast of Brazil. Campina Grande. Rev. Bras. Eng. Agrí. Ambient, 11:537-531.

Lammers, P., B.J. Kerr, T.E. Weber, W.A. 3rd Dozier, M.T. Kidd, K. Bregendahl, and M.S. Honeyman (2008). Digestible and metabolizable energy of crude glycerol for growing pigs. Champaign. J. Anim. Sci. 86:602-608.

Marchini, C.F.P., P.L. Silva, M.R.B. Nascimento, and M. Tavares (2007). Respiratory frequency and cloacal temperature in broiler chickens submitted to high cyclic ambient temperature. Paraná. Arch. Vet. Sci. 12:41-46.

Melotti, V.D., G.B. Aguiar, J.A. Brumatti, and S.S. Morais (2011). Influence of comb and wattle on body thermoregulation of birds. Literature Review. Available at: <http://webartigos.com/artigos/influencia-da-crista-e-barbela-na-termorregulacao-corporal-de-aves/69371>. Accessed February 2016.

Menten, J.F.M., P.W.Z. Pereira, and A.M.C. Racanicci (2008). Evaluation of the biodiesel-derived glycerin as an ingredient for broiler diets. **In:** Conferência APINCO 2008 de Ciência e Tecnologias Avícolas, Santos, SP. Proceedings… Campinas: Fundação APINCO de Ciência e Tecnologia Avícolas, 66.

Nascimento, S.T. and I.J.O. Silva (2010). Heat losses in poultry: understanding heat exchanges with the environment. Literature Review. 1-5. Available at: <http://www.avisite.com.br/cet/img/20100916_trocasdecalor.pdf>. Accessed February 2016.

Passini, R., M.A.G. Araujo, V.M. Yasuda, and E.A. Almeida (2013). Environmental intervention in roof covering and artificial ventilation on the comfort indices for broiler. Campina Grande. Rev. Bras. Eng. Agrí. Ambient. 17:333-338.

Romano, G.G., J.F.M. Menten, L.W. Freitas, M.B. Lima, R. Pereira, K.C. Zavarize, and C.T.S. Dias (2014). Effects of Glycerol on the Metabolism of Broilers FED Increasing Glycerine Levels. Campinas. Rev. Bras. Cienc. Avi. 16:97-106.

Rosa, J.F.V. (2009). Evaluation of porous panels made of expanded clay in a evaporative adiabatic cooler systems. Dsc. Thesis. Federal Univ. of Viçosa, Viçosa, MG.

Rostagno, H.S., L. F. T. Albino, J. L. Donzele, P. C. Gomes, R. F. Oliveira, D. C. Lopes, A. S. Ferreira, S. L.T. Barreto, and R. F. Euclides (2011). Brazilian Tables for poultry and swine: composition of feedstuffs and nutritional requirements. 3rd Ed. UFV. Viçosa (Brasil). 3:1-252.

SAS ®. 2000. User´s Guide: Statistics, Version 10th. SAS Institute Inc. Cary, NC.

Sousa, A.M., T.L. Sena, A.M. Vasconcelos, and S. C. Bastos-Leite (2016). Comb and wattle temperatures as indicators in the thermoregulation of broilers reared in the semi-arid region of the municipality of Sobral – CE. **In:** XXV Congreso de la Asociación Latinoamericana de Produción Animal e XI Congresso Nordestino de Produção Animal. 2016, Recife - Pernambuco, Brasil.

Tinôco, I. F. F. (2001). Industrial Aviculture: New Concepts of Materials, Conceptions and Constructive Techniques Available for Brazilian Poultry Houses. Campinas. Rev. Bras. Cienc. Avi. 3:1-26.