



Welfare of Broilers Ingesting a Pre-Slaughter Hydric Diet of Lemon Grass

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ABSTRACT

The pre-slaughter period is considered critical in broiler production. Several factors contribute to increase the birds' stress, such as handling, harvesting, and transportation, negatively affecting their welfare. This study aimed at evaluating the addition of lemon grass (*Cymbopogon citratus* Stapf) to the drinking water of broilers during the pre-slaughter period on their behavior, blood cortisol, and surface temperature. The study was carried out at the experimental farm of the Federal University of Grande Dourados (UFGD), Dourados, MS, Brazil. In total, 2594 broilers were distributed according to a completely randomized experimental design, in a 3x2x2 factorial arrangement, with four replicates per treatment. Treatments consisted of three different lemon grass levels (*Cymbopogon citratus* Stapf) used in the form of an infusion (0, 0.1, and 5 g per L of water), sex (male or female), and genetic strain (Ross® 308 or Cobb® 500). The infusion was offered when birds were 42 days old. On that day, blood was collected for blood cortisol level determination, broiler surface temperature was recorded, and an ethogram was applied to register broiler behavior. Blood cortisol level and broiler surface temperature were not affected by treatments ($p > 0.05$). The behavior of beak opening was different between the genetic strains ($p < 0.05$), being more frequent in Ross® 308 broilers. Lemon grass water content did not affect broilers' surface temperature when consumed during the pre-slaughter period.

INTRODUCTION

During the pre-slaughter period before harvesting, broilers are kept at high densities, and are submitted to stresses, such as feed withdrawal, poor ventilation in the transport crate, and handling and harvesting handling practices (Barbosa Filho, 2012). This management negatively affected broiler welfare and interferes with their corticosterone balance (Macari & Luqueti, 2002), which is a hormone released due to stress exposure. Corticosterone may also induce cardiovascular diseases, ascites, and modifications in immune functions (Grandin, 1998), in addition of causing lymphoid tissue hypotrophy and hypoplasia (Compton *et al.*, 1990). In addition of blood changes, an increase in the heat exchange in the featherless body parts of broilers under stress is also observed (Dahlke *et al.*, 2005) due to the increase in peripheral vasodilatation (Borges *et al.*, 2003), leading to variation in surface temperature (Nääs, 2011).

Infrared technology is a valuable tool to evaluate heat exchange in broilers as it accurately records surface temperature (Cangar *et al.*, 2008; Yahav *et al.*, 2008), allowing the estimation of metabolic heat exchange, which is associated with heat stress.



Another key aspect in the estimation of broiler welfare is behavioral observations (Pereira *et al.*, 2007), including the analysis of their social behavior. It is possible to infer on broiler welfare status is a flock by determining the quantity and quality of social interactions, (Nääs *et al.*, 2005; Nazareno *et al.*, 2011).

Some plants contain chemical active principles that may reduce anxiety and stress in humans (Rocha *et al.*, 2008). Lemon grass (*Cymbopogon citratus* Stapf) affects the sympathetic nervous system, reducing stress (Negrelle & Gomes, 2007). Although some researchers investigated the use of phytotherapy in poultry production, most are related to animal nutrition (Wenk, 2003; Hashemi & Davoodi, 2010) and microbiological control (Arshad *et al.*, 2008; Lourenço *et al.*, 2013). This study aimed at evaluating the use of lemon grass (*Cymbopogon citratus* Stapf) to reduce broiler pre-slaughter stress.

MATERIALS AND METHODS

This study was carried out at the experimental poultry sector of the Federal University of Grande Dourados, MS, Brazil, and it was approved by the Ethics Committee (CEUA; 007/2012). An open-sided poultry house (50-m long; 10-m wide, and 3-m high) was divided into 56 pens measuring 4.5 m² each. Each pen was equipped with a bell drinker and a tube feeder. The house environment was controlled by the use of side double curtains and ventilation fans and foggers distributed throughout the house, as well as an evaporative cooling system controlled by a software.

The experiment was carried out in September, 2012. The birds were reared according to the genetic strain manual, at a final flock density of 16 birds/m². House environment (dry bulb temperature and relative humidity) was maintained as recommended by the genetic strain manual. Litter consisted of rice hulls (5-cm deep). Broilers were reared until 42 days old and were offered water and feed *ad libitum*. Commercial balanced feeds, supplying the broilers' requirements for the pre-starter, starter, grower, and finisher phases, were fed.

In total, 2592 42-d-old broilers were distributed according to a completely randomized experimental design in a 3x2x2 factorial arrangement, with four replicates of 54 birds each. Treatments consisted of three different levels of a lemon grass (*Cymbopogon citratus* Stapf) infusion added to the drinking water (0, 0.1, or 5 g/L of water), two sexes, and two genetic strains (Ross® 308 e Cobb® 500).

The oven-dried lemon grass (*Cymbopogon citratus* Stapf) leaves were acquired from a commercial company (Flores e Ervas Comércio Farmacêutico Ltda., São Paulo, Brazil). The infusions (0, 0.1, or 5 g of leaves/L of water) were prepared by crushing the dried leaves and immersing them in boiling water (100° C). The infusions were left to cool until reaching room temperature, filtered, and then poured into the drinkers (Cruz *et al.*, 2007). The infusions were offered to the broilers during the 6-h pre-harvesting fasting period (Castro *et al.*, 2008) on the 42nd day of the grow-out. The infusion was made available to the birds starting at 02h:00 min. During the same period, ambient temperature (Ta) was recorded using a digital thermo hygrometer HT-200 (Instruterm, Company, SP-Brazil).

Thermal images were recorded three times during the trial using an infrared camera (Testo®, model 880) with an accuracy of ± 0.1 °C and a series of 7.5 µm of the infrared spectrum, placed at 1 m height from the birds. Images were recorded one hour after the infusions were offered (03h:00min), at 05h:00min, and 07h:00min. Two thermal images per treatment, per replicate, and per recording time were analyzed using a thermal-imaging software (IR-Soft, Testo®, 2009), with iron filter and emissivity coefficient of 0.95 for the broiler body surface (Nääs *et al.*, 2010). In order to accurately determine broiler surface temperature (Ts), 30 points were randomly selected on the broiler body, and the average value was calculated. Temperature data were submitted to analysis of variances and means were compared by the test of Tukey at 5% significance levels, using R Statistical Software (2012).

Broiler behavior was evaluated by indirect monitoring using video footage. A total of three video-recordings (5 min long each) were made per treatment. Three broilers were selected for observation during the total video footage, and the video was played and re-played until each bird was observed for 5 min. Broiler behaviors (Table 1) were observed and recorded as suggested by Souto (2003). The activities were computed in percentage within the observational time, with 5 min equivalent to 100%. The results were submitted to analysis of variance and means were compared by the Kruskal-Wallis test at 95% of significance level, c (2012).

Twelve broilers per treatment were selected (three per replicate, as recommended by Rosa *et al.*, 2002). Birds were duly identified and placed in transport crates (10 birds per crate) and transported to the Meat Laboratory of the Federal University of Grande Dourados (UFGD). Before slaughter, 5 mL of blood were collected per



Table 1 – Descriptive ethogram of the experimental observed behaviors

Behavior		Description
Drinking	BE	The bird stays at the drinker and ingests water.
Pecking other bird	BF	The bird aggressively pecks any part of the body of another bird.
Preening	P	The bird arranges its feathers with the beak, stimulating the uropigeal gland.
Standing still	PP	The bird is standing up and observing its surroundings.
Walking briskly	AR	The bird walks rapidly among the other broilers.
Standing or walking slow	AD	The bird moves slowly among the other broilers.
Lying down/sleeping	D	The bird either sits or lays down on the litter and sleeps.
Lying attentive	DO	The bird either sits or lays down on the litter and notes.
Lying on its side	DL	The bird either sits or lays down in lateral recumbency.
Opening the beak	AB	The bird sits on the litter with open beak.
Stretching	AA	The bird stretches one wing and one leg of the same body hemisphere.
Flapping the wings	BA	The bird flaps both wings.

bird by ulnar vein puncture in order to quantify blood cortisol levels (Valle *et al.*, 2008). Blood samples were collected in test tubes containing one drop of heparin (15 µL heparin/1 mL blood) and identified per treatment. The tubes were centrifuged at 4000 rpm for 10 min (CentriBio, 80-2B, SP-Brazil) to, and the supernatant was homogenized, resulting in three samples per treatment. Samples were stored at 20°C (Valle *et al.*, 2008) and analyzed using a commercial kit (Cortisol EIA, E52061 Westbrook, ME, USA).

The results were compared using the Kruskal-Wallis test at 95% of significance level, using R Statistical Software (2012). Cortisol levels were converted from nmol/L (titration unit) to µ/dL of blood using a correction factor of 27.59.

RESULTS AND DISCUSSION

Broiler surface temperature was not affected by sex, genetic strain, or lemon grass infusion levels ($p > 0.05$) during the pre-slaughter fasting period (Table 2). Air ambient temperature and relative humidity (RH) measured each time the birds' surface temperature was recorded were: recording 1= 20.5° C and 58.9 RH; recording 2 = 22.0 °C and 52.1 RH; recording 3 = 24.0°C and 51.3 RH. No interaction was found between the times infrared images were recorded and broiler surface temperature, differently from results of Santi (2012). This author, using infrared imaging, observed lower broiler surface temperature at the times when environmental temperature were lower (17.9° C; 20.0° C; 25.7° C), confirming the relationship between body and environmental temperatures. According to Borges *et al.* (2003), broilers become sensitive to heat stress when air relative humidity and ambient temperature exceed the thermal neutral zone values, hindering heat dissipation and increasing their body temperature to hazardous limits. This has

a negative effect of live performance and may result in death. The recommended ambient temperature and air relative humidity for broilers are 21-23° C and 65-70 %, respectively (UBA, 2008).

Table 2 – Average body surface temperature of broilers as a function of sex, genetic strain, and lemon grass (*Cymbopogon citratus* Stapf) levels in the drinking water.

Treatment	Temperature ° C		
	20.5 ° C	22 ° C	24° C
Sex			
Male	34.6	34.0	35.7
Female	33.8	33.3	35.1
Genetic strain			
Ross®308	34.2	33.7	35.4
Cobb® 500	34.5	33.6	35.3
Amount of lemon grass			
0	34.1	33.8	35.2
1	34.1	33.5	35.5
5	34.4	33.7	35.4
CV (%)	6.97	5.97	5.33

Means compared by the test of Tukey at 95% significance level.

Ross® 350 broilers exhibited higher frequency of open beak behavior ($p < 0.05$) than Cobb® 500 broilers. This behavior was previously described by Pereira *et al.* (2007), who reported that Ross® 350 broilers were more susceptible to stress than Cobb®500 broilers, and may present high metabolic rates when faced with stressful situations, such as pre-slaughter handling (Roque-Specht *et al.*, 2009). Broilers usually change their behavior when facing stress, and try to adapt to it (Dahlke *et al.*, 2005). Some behavioral patterns found in the present study (Figure 1) were also reported by other authors, such as wing flapping and stretching (Alcock, 2011). However, these are kinetic activities and may not be associated with stress (Morrison,

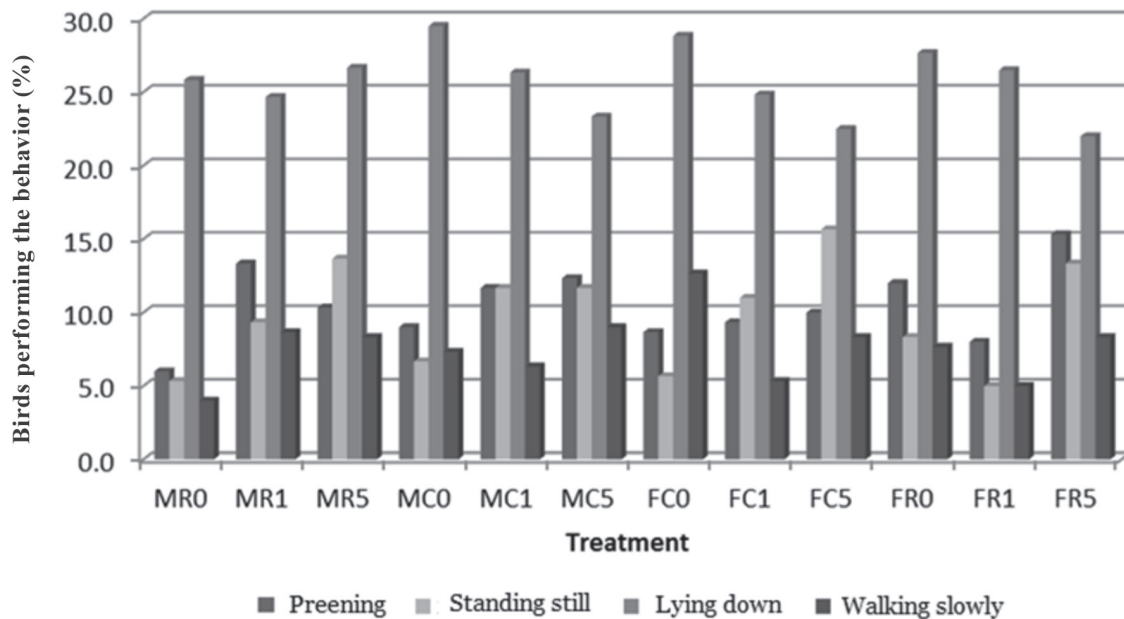


Figure 1 – Most frequently observed behaviors of broilers offered different levels of lemon grass in the drinking water during the pre-slaughter period.

2007). Except for pecking other birds, no negative interactions among birds were observed during the observation period (Table 3). Preening is described as the behavior of cleaning and tiding its feathers with the beak (Pereira *et al.*, 2013), and it is an indication of normal behavior. It was observed in several birds of all groups during the observation period; however, no differences were found among treatments (Figure 1).

Blood cortisol levels were not different between sexes or genetic strains. In addition, lemon grass levels did not influence blood cortisol levels ($p>0.05$), which

is consistent with the findings of Quinteiro Filho (2008). Several factors may increase blood cortisol levels when birds are exposed to stressors (Macari *et al.*, 1994; Caires *et al.*, 2008). In the present experiment, it was expected that, due to its known phytotherapeutical effect of reducing human anxiety, the lemon grass infusion would be able to reduce broilers blood cortisol levels. However, the results indicate that the active chemical principles of lemon grass were not able to change blood cortisol levels of broilers during the pre-slaughter period.

Table 3 – Overall behavior of broilers offered different levels of lemon grass (*Cymbopogon citratus* Stapf) in the drinking water during the pre-slaughter period.

Treatment	Behavior											
	AA	AB	BA	BE	BF	DL	D	DO	P	PP	AR	AD
Sex												
Male	0.9	1.4	1.8	0.5	2.4	5.9	0.2	0.4	0.2	0.6	1.8	0.1
Female	1.0	0.8	1.8	0.5	2.4	5.5	0.2	0.2	0.2	0.6	1.9	0.1
Genetic strain												
Ross®308	0.7	1.4 a	1.7	0.7	2.4	5.5	0.3	0.4	0.1	0.5	1.9	0.1
Cobb®500	1.2	0.7 b	1.9	0.3	2.4	5.9	0.2	0.2	0.3	0.7	1.8	0.2
Lemon grass level												
0	0.5	1.2	1.3 b	0.3	2.8	5.3	0.2	0.4 ab	0.2	0.6	1.5	0.1
1	1.3	1.1	1.7ab	0.7	2.7	5.5	0.2	0.5 a	0.2	0.6	1.8	0.2
5	1.2	1.0	2.4 a	0.4	1.7	6.3	0.2	0.03 b	0.2	0.5	2.2	0.2

Means followed by different letters in the same column are different by the Kruskal-Wallis test with 95% significance level. The abbreviation of the behaviors is presented in Table 1.



CONCLUSION

There was no effect of lemon grass infusion on plasma cortisol levels, surface temperature, or behavioral traits of broilers evaluated during pre-slaughter fasting periods. Studies on the stress of broilers offered lemon grass during the pre-slaughter feed-fasting period are needed.

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