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Broiler Pre-Slaughter Water Diet with Grass Lemongrass (Cymbopogon Citratus Stapf)

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ABSTRACT

The pre-slaughter management is one of the predisposing factors to the reduction in the quality of the carcass and broiler meat, mainly for being a stressful condition. This study evaluates the inclusion of lemongrass (*Cymbopogon citratus* Stapf) in the water used in the diet of pre-slaughter broilers for the first time. The carcass and meat quality parameters were evaluated. The experiment was carried out in the poultry production of an experimental sector of the Federal University of Grande Dourados – UFGD. A total of 2,594 broilers were distributed in an entirely randomized design in a factorial arrangement of 3x2x2, with three different lemongrass concentrations in the form of infusion (0. 1 and 5 g/L), two sexes and two genetic strains (Ross 308® and Cobb 500®), and with four replications. After 42 days, 144 broilers were slaughtered, and the quality parameters of carcass and meat were evaluated. A higher incidence of scratches and higher water retention capacity were found in Ross 308® male (p<0.05). Less exudate of breast fillets loss was observed in broiler Ross 308® males 72 h *post-mortem* (p<0.05). There was an interaction between sex and lemongrass levels in the drinking water of the broilers in the sensory analysis of meat (p<0.05), more preferably of chewiness and juiciness for males undergoing free diet for juiciness and lemongrass in the female diet with 5g/L. The use of lemongrass infusions in pre-slaughter did not bring considerable benefits to the quality of carcass and meat of broiler.

INTRODUCTION

The pre-slaughter handling is characterized by catching and transportation of broilers. It is a stressful situation for the animals, with the release of fear and panic reactions that interfere with the welfare conditions and, in some cases, results in economic losses, such as the increase in mortality levels and the incidence of scrapes in the carcass (Paranhos da Costa, 2008). Carcass quality can be affected by various aspects such as age, sex, nutrition, management, temperature, time of fasting, and methods of harvesting and transportation (Mendes & Komiyama, 2011). Issues during collection and transportation might be directly related to the animals' welfare and might lead to abnormal biochemical changes during the transformation of the muscle meat. As a consequence, it results in the acceleration of glycolysis reactions altering the quality of characteristics and promoting the occurrence of meat PSE (*Pale*, *Soft*, *Exudative*) with a pale, soft and exudative appearance (Bressan & Beraquet, 2002).

As a result, there is a need for studies aiming at reducing the impact of pre-slaughter and the adoption of low-cost management practices, offering sustainable alternatives to the producers. Several plants might affect the behavior and the mechanisms of action of individuals, in which anxiety and stress disorders are the main factors for researching and for

the development of new pharmacotherapies (Rocha *et al.*, 2008). From their extracts and constituents, it might be possible to find positive effects on the welfare relationship and poultry production on an industrial scale. Some plants have anxiolytic potential and are widely used in human therapeutic. The grass lemongrass (*Cymbopogon citratus* Stapf) is a species of medicinal plants commonly used in folk medicine in Brazil for its calming and sedative purposes and action on the central nervous system. However, in animal production research it is practically nonexistent (Negrelle & Gomes, 2007). This study aimed to evaluate the use of lemongrass the in water of broiler diets during the pre-slaughter on carcass and meat quality parameters.

MATERIAL AND METHODS

This study was carried out in the Experimental Poultry Sector of the Federal University of Grande Dourados – UFGD using 2594 broilers. The birds were distributed in an entirely randomized design in a factorial arrangement of 3x2x2, with three different levels of dried and ground lemongrass grass (*Cymbopogon citratus* Stapf*)* in the form of infusion (0, 1 and 5 g of dry powder L water). The beverage was tested in two sexes, and two lineages (Ross 308® and Cobb 500®) with four replicates with 54 broilers. The broilers were conventionally grown according to the manual of the genetic strain under study, and the supply of grass lemongrass infusions in different concentrations was performed *ad libitum*, during the fasting of the birds before slaughter at 42 days of age.

Infusions of lemongrass (*Cymbopogon citratus*) were prepared from the immersion of dried and ground grass in water at a temperature of 100°C and left to rest until complete cooling, with subsequent filtering (Cruz *et al.*, 2007). The infusions were administrated to the broilers during the fasting during six hours. Subsequently, twelve broilers were separated per treatment, properly harvested by the back, identified, and accommodated in the transport boxes and sent to the Meat Technology Laboratory. Conventional slaughtering procedures in the poultry industry were adopted, and whole and hot carcasses were evaluated for the presence and absence of gathering, transporting and growing scratches (Pilecco *et al.*, 2011). Subsequently, the breast sections were deboned, separating the *Pectoralis major*, and other muscle samples were kept under refrigeration (2°C) to the achievement of the quality analysis. The assessment of the pH of the broiler breast fillets was performed at 4, 12, 24 and 48 hours *post-mortem* using a pH-meter (Hanna bench pH-meter, pH 21 Model pH/MV) calibrated with two standards (pH 4.0 and pH 7.0). An incision was made at the top of the sample for the introduction of the electrode.

Table 1 – Colorimetry *Pectoralis major* muscle broiler subjected to water diet with lemongrass (*Cymbopogon citratus* Stapf) in the form of infusion in three concentrations.

Treatment	Colorimetry		
	a*	b*	L*
Gender			
Male	12.2	19.4	55.9
Female	12.1	20.6	56.8
Geneticstrain			
Ross 308®	12.2	19.6	56.1
Cobb 500®	12.1	20.3	56.6
Level			
0	12.0	20.2	56.4
1	12.3	20.1	56.1
5	12.1	19.6	56.6
CV%	0.08	0.13	0.11

Means were compared by the Tukey test using 95% confidence level.

The evaluation of the loss of exudate was performed using six breast meat samples by treatment, kept under retailing sale simulation in polyethylene trays covered with a plastic film, the 3 ± 1°C for 24, 48 and 72 h. From each tray, exudate was discarded, and samples were weighed on an analytical balance, maintaining the trays and weighing samples for the following time. The loss of exudate was obtained by the difference between the initial and final weights of samples and expressed as percentage loss in the initial sample weight. The water retention capacity was done following the method described by Hamm (1960), by evaluating six breast meat samples with 24, 48 and 72 h *post-mortem.* The process is done by removing two grams of each sample meat in the form of cubes and placed between two circular filter paper then placed between two glass plates which received 10 kg for five minutes to determine the quantity of water released under pressure weight on muscle tissue. After pressing, the sample was weighed, and the result expressed in percentage of water exuded from the initial sample weight.

The color of the broilers fillets was measured using portable colorimeter Minolta CR 400 model, with results shown in CIELab system, light source D65 and angle of 10°. The evaluation was done on the lower part of the muscle Pectoralis major after the removal of the *Pectoralis minor.* The values of L * (lightness), a * (red intensity) and b * (yellow intensity) were assessed measured at two different places in the lower ventral

region of the muscle, by calculating the average of the values obtained.

The weight loss on cooking was determined to six broilers breast fillets by treatment, weighed on an analytical balance and baked in an electric oven at 180°C for 16 minutes (eight minutes/side) until reaching an inside temperature of 82°C, measured with digital thermometer skewer. Later they were cooled at ambient temperature and weighed and the difference between the initial and final weight corresponded to the cooking weight loss expressed as percentage loss compared to the initial weight. The cooked fillets were used to determine the shear force, by removing three cubes of 2 x 2 x 1.13 cm of the breast fillet and placing them in the texture meter device (TAXT 2i Stablemicro Systems). The fibers were oriented in a perpendicular direction to the Warner-Blatzler blades of the apparatus, measuring up the strength to cut them with a value expressed in kgf for cm^2.

The sensory evaluation of meat was done at the Agricultural Sciences in Animal Products Technology Laboratory applying sufficient proof through the acceptance test with verbal hedonic scale, numeric, bipolar five-point, with the participation of 41 inexperienced tasters. The evaluated attributes were the softness (texture to the touch and chewing), the color (appearance) and the acceptance (odor, juiciness, and flavor) getting scores about the approval of the tester through inductive questionnaire activity (Zenebon *et al.*, 2008).

The meat samples were distributed in six treatments, based on the results obtained by Takahashi *et al.* (2012) who used only the factors of sex and lemongrass levels for the evaluation, and eliminated the role of genetic strain on the sensory characteristics. Therefore, fatigue was avoided by too many tests and maintaining an appropriate number of repetitions (Torres *et al.*, 2011). The breast samples previously kept in a freezer (-18 °C) were unfrozen, wrapped with aluminum foil and taken to the oven until they reached an internal temperature of 82 °C measured with a digital thermometer. After cooking, the meat was cut into cubes measuring 1 x 1 cm (Torres *et al.*, 2011). The pieces were packed in foil and heated for the tasters, instructed to point at the scale of 1 to 5 the samples that showed better characteristics for the evaluated parameters, considering 1 for the worst sample and 5 for the best one (Zenebon *et al.*, 2008).

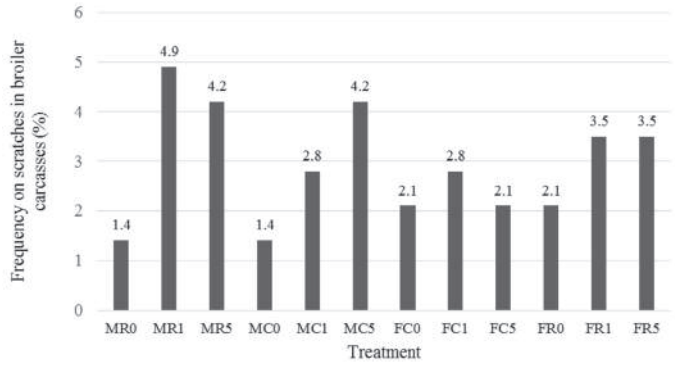
The model of the analysis assumption (data additivity, homoscedasticity, and normality) was performed on the results and tested for all traits. Those features that met the assumptions were analyzed using the one-way analysis of variance (ANOVA). The means were compared using the Tukey test for the carcass and meat characteristics. Those elements which did not meet the suppositions were treated as non-parametric and further analyzed using the Kruskal-Wallis test (Delgado-Suárez *et al.*, 2016). Data were processed using the Software Core Team® (Core Team R, 2012). Reported differences were significant at the 0.05 level.

The research was approved by the Ethics Committee on Animal Use of UFGD with protocol number 007/2012.

RESULTS

No interactions were found amongst the factors sex, lineage, and lemongrass levels; however, there was a higher incidence of scratches on the carcasses of males, and a greater frequency of scratches to the genetic strain Ross 308® ($p<0.05$) (Figure 1). Amongst the tested lemongrass levels, the incidence of scratches varied between treatments, not ensuring the influence of a degree of quality of carcasses, and the presence of bruising in carcasses was not observed. However, broilers which received the infusion had a lower incidence of unhealed scratches (Figure 1). The pH values did not differ ($p>0.05$) between treatments obtaining average values between 5.9 and 6.3. The *L**, *a**, and *b** meat color parameters were similar in the treatments ($p>0.05$).

Figure 1 – Results of the frequency of scratches in broiler carcasses.



(MR0 - Males Ross 308® / 0g; MR1 - Males Ross 308® / 1g; MR5 - Males Ross 308® / 5g; MC0 - Male Cobb 500® / 0g; MC1 - Male Cobb 500® / 1g, MC5 - Males Cobb 500® / 5g; FC0 - Females Cobb 500® / 0g; FC1 - females Cobb 500® / 1g; FC5 - females Cobb 500® / 5g; FR0 - females Ross 308® / 0g; FR1 - Female Ross 308® / 1g ; FR5 - Ross Female 308® / 5g).

There was no difference among treatments for water retention capacity of the fillets 48 h *post-mortem* ($p>0.05$). However, the genetic strain interfered with

the water retention capacity of 72 h *post-mortem* (p<0.05) higher water retention capacity obtained in the genetic strain Ross® 308 (Table 2). The lemongrass levels in drinking water and the genetic strain of the broiler did not affect the weight loss by exudation of meat (p>0.05). However, regarding sex, the male breast fillets showed less weight loss exudate when compared to females at 72 h *post-mortem*. There was no effect of treatments on weight loss by cooking (p>0.05), and shear strength of the chest fillets evaluated (Table 2).

Table 2 – Loss of exudate (PE), water holding capacity (WHC), weight loss by cooking (PPC) and shear force (FC) muscle *Pectoralis major* broiler subjected to water diet with lemongrass (*Cymbopogon citratus* Stapf).

Treatment	PE (%)			CRA (%)		PPC (%)	FC (kgf/cm²)
	24h	48h	72h	48h	72h		
Gender							
Males	1.8	2.7 b	4.5 b	38.1	36.4	19.8	2.1
Female	2.1	3.5 a	5.3 a	38.5	31.7	19.1	2.3
Geneticstrain							
Ross 308®	2.1	3.1	5.2	39.1	37.6 a	20.4	2.1
Cobb 500®	1.8	3.2	4.7	37.6	30.5 b	18.5	2.1
Level							
0	2.0	2.9	4.9	31.4	36.6	20.2	2.0
1	2.1	3.3	4.8	41.8	32.9	19.8	2.2
5	1.7	3.2	5.1	41.8	32.1	18.4	2.3
CV%	38.0	9.9	13.5	35.2	33.8	0.8	0.3

Means with different letters in the column are significantly different by Tukey test (p< 0.05).

There was an interaction between sex and lemongrass levels in the broilers drinking water on the sensory analysis of the meat (p<0.05), except for variable flavor (Table 3). Fillets of males showed the influence of lemongrass levels on chewiness and juiciness of the meat (p<0.05), observing best chewiness and juiciness in samples from broilers that did not receive the lemongrass in pre-slaughter. The meat from the

Table 3 – Sensory analysis *Pectoralis major* muscle broiler submitted to water diet with lemongrass (*Cymbopogon citratus* Stapf) in the form of infusion in three concentrations.

Appearance				
Gender	0	1	5	Average gender
Female	3.3	3.4	3.6	3.3
Male	3.5	3.2	3.1	3.4
Average Level	3.4	3.3	3.3	-
Fragrance				
	0	1	5	Average gender
Female	3.5	3.7	3.4	3.5
Male	3.6	3.5	3.7	3.6
Average Level	3.6	3.6	3.6	-
Chewiness				
	0	1	5	Average gender
Female	3.8	3.8	3.9	3.8
Male	4.3 a	3.7 b	3.6 b	3.9
Average Level	4.1	3.8	3.8	-
Succulence				
	0	1	5	Average gender
Female	3.2 b	3.2 b	3.7 a	3.4
Male	3.7 a	3.1 b	2.8 b	3.2
Average Level	3.5	3.2	3.3	-
Flavor				
Gender		Male	Female	
		3.6	3.6	
Level	0	1	5	
	3.6	3.5	3.6	

Means with different letters in the line are significantly different by Kruskal-Wallis test (p< 0.05).

females which drank the lemongrass infusion of 5g per L presented greater juiciness (p<0.05) than the other meat tested. No difference was identified between the sexes and lemongrass levels when tested isolated (p>0.05).

DISCUSSION

The results obtained in the incidence of scratching on the carcasses of males agree with the results achieved by Pilecco *et al.* (2011). The author related a high frequency of injuries and the final weight difference between the sexes of broilers, since the females have lower mass than the males, and getting more space in the rearing and transport, besides being less disposed to cramping. The weight difference between the sexes has been reported in the literature, especially from the 21st of grow-out (Santos *et al.*, 2005). The highest incidence of scratches on Ross 308® genetic strain might be justified by the different behavior shown by the genetic strains when in stressful situations. Ross 308® broilers exposed to high temperatures in a study developed by Pereira *et al.* (2007) were more active than those from the Cobb 500® genetic strain. This behavior might occur because of the metabolic acceleration seen in broilers in stressful conditions (Roque-Specht *et al.*, 2009). Thus, the increase of scratches on Ross 308® can be related to the stacking of broilers during transportation within boxes in search for the sides for a more efficient thermal exchange.

The absence of bruising on the carcass might be related to the transport period used in the present study, when the boilers were carried out in the morning. According to Barbosa Filho *et al.* (2009), during the morning and the night shift, a significant region of thermal comfort throughout the load profile was observed. Thus, the best time for transportation of broilers is during the morning and night shifts since this period of the day provide more favorable environmental conditions for the pre-slaughter operation.

The average pH values between 5.9 and 6.3 are close to the values of 5.94 and 6.01 found by Pavan *et al.* (2003). The pH values below 5.6 might cause the development of meat with PSE characteristics (pale, soft, exudative) in chickens. A rapid decrease in pH causes denaturation of myofibrillar proteins and sarcoplasmic leading to excessive loss of exudate and resulting in pale meat by the increase of the birefringence of muscle fibers (Droval, 2011). Therefore, the pH isolated is not alone efficient to diagnose PSE in meat, but the association with the color, loss of exudate and water capacity retention in meat (Lara *et al.*, 2003).

Similar results to those found in the present work for the values of L*, a*, and b* were described by Santos *et al.* (2005) where the genetic strain and sex also did not interfere in the meat color parameters. The color can be influenced by several factors such as enzymes, diet, the age of the animal and events before slaughter. The pallor is associated with protein denaturation caused by low pH and high carcass temperature (Droval, 2011). According to Fragoso (2012), the meat surface color is the result of selective absorption of wave lengths of light that emerges from the meat with the natural pigments and the meat fibers. This characteristic is dependent on the amount of heme pigment (myoglobin, hemoglobin, and cytochrome C) absorbsion of all track colors except the range between 630-780 nanometers, corresponding to red, which is reflected in the eyes (Cornforth, 1994; Praxedes, 2007). By decreasing pH, protein denaturation and expulsion of water, the protein-protein interaction increases and the occurrence of the birefringence of the fibers with less light being transmitted through the meat and more light is scattered due to heme pigment susceptibility to chemical changes, modifying the capacity to absorb the incident light rays present in the meat. Thus, there is a reduction of the perception of red in the meat causing paleness (Fragoso, 2012).

The water retention capacity is related to the sudden fall of the pH during glycolysis *post-mortem* (Ribeiro, 2008). According to Maganhini *et al.* (2007) physical exercise, transportation, movement and prolonged fasting involves the consumption of the glycogen reserves, leading to slow glycolysis with the relatively increased formation of muscle lactic acid and a slight reduction in pH in the first hours. There is a subsequent stabilization that remains at higher levels 6.01. This fact makes the muscle proteins retain a great capacity to keep water inside the cells. This effect justifies the water retention capacity presented in Ross 308® broilers considering the little ability to adapt to stressful situations and greater movement during catching and transportation when compared to Cobb 500® (Pereira *et al.*, 2007). Results of Mollete *et al.* (2003) showed broilers breast cuts considered normal with a water retention capacity greater than 62.5%. The values obtained for water retention capacity of the present study are below these values, as well as those obtained by Huallanco (2005) (54%). However, it does not characterize PSE cuts, since the original classification depends on pH values of less than 5.6, which was not observed in this study.

Distinctly perceived in the present study, Moreira *et al.* (2003) found no influence of sexes on the quality

of meat of different lineages evaluated. Schneider *et al.* (2005) describe a loss of normal exudate cuts of 2.55%, but as the weight loss by cooking and shear strength, the loss of exudate muscle is related to water retention capacity. However, regarding the genetic strain effects, the results obtained agreed with those presented by Mendes & Komiyama (2003). The authors also found no effect of the genetic strain on meat quality parameters and the results are similar to those observed by Pavan *et al.* (2003) and 19% is below the threshold values that define the PSE meat (Schneider *et al.*, 2005) for losses above 25%.

The results found for weight loss by cooking shearing force differ to the ones observed by Santos *et al.* (2005) who noted the influence of genetic strain on the shear strength of the meat. Pavan *et al.* (2003) describe shear force limits to soft broiler breasts meat within a range of variation between 1.91 and 2.23 kgf per cm^2. Higher values for shear strength, as found by Huallanco (2005) (3.0 to 8.2 kgf per cm^2), might be associated with the factors that cause stress in broilers during the *antemortem*, such as time and distance of transport between the farmhouse and the slaughterhouse.

Individual organoleptic characteristics such as texture and flavor of meat are more influenced by age than by other factors such as weight, genetic or sex (Takahashi *et al.*, 2012). The definition that diverges from results reported by Ricard & Touraille (1988) cited by Julião (2003), where the pectoral muscle of females was more tender than in males, and the flavor of the meat was more prominent in male broilers.

CONCLUSIONS

The use of lemongrass infusion in water pre-slaughter diet did not affect the carcass quality, and broiler meat neither changed the stressful condition of broilers.

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