Physiological responses of broiler chickens following controlled atmosphere or electrical waterbath stunning

Ileana María Berganza Portillo

Escuela Agrícola Panamericana, Zamorano
Honduras
November, 2019

ZAMORANO
FOOD SCIENCE AND TECHNOLOGY MAJOR

Physiological responses of broiler chickens following controlled atmosphere or electrical waterbath stunning

Special graduation project presented as partial requirement to obtain the Food Science and Technology Bachelor Degree.

Presented by

Ileana María Berganza Portillo

Zamorano, Honduras
November, 2019

Physiological responses of broiler chickens following controlled atmosphere or electrical waterbath stunning

Ileana María Berganza Portillo

Abstract. The poultry industry has evolved and cares more for animal welfare looking for stunning options. Our research goal was to assess the potential for CAS systems for Halal slaughter. The impact of a multiphase CAS system (Controlled Atmosphere Stunning) on blood loss and cessation of heartbeat was assessed in broilers stunned by four methods: 1) electric water bath, 2) multiphase CAS withO_2 added during the induction phase, 3) CAS multiphase without O_2 added and 4) without stunning. The birds were equipped with cutaneous electrodes (ECG) registered with a PhysioTel DSI device, a neck cut was performed on all birds. The data were analyzed by analysis of variance (ANOVA), and a Pearson correlation was made between the EGG and the blood loss according to the sacrifice methods. Non-stunned or electrically stunned birds lost more blood compared to CAS birds with or without O_2. At 10 seconds, there was a statistical difference for blood loss. The interruption of the heartbeat occurred more rapidly for non-stunned and electrically stunned birds compared to CAS with or without O_2. For CAS birds, the application of the cut in the neck did not impact the cessation of the heartbeat. The use of CAS did not alter the cessation of heartbeat compared to birds not stunned or electrically. Both traditional and CAS methods can be used in Halal markets.

Keywords: Blood loss, Electrocardiogram, halal, sacrifice.

Resumen. La industria avícola ha evolucionado y se preocupa más por el bienestar animal, buscando alternativas de aturdimiento. Nuestro objetivo de investigación fue evaluar el potencial de los sistemas CAS para el sacrificio Halal. Se evaluó el impacto de un sistema CAS (aturdimiento con atmosferas controladas) multifase en la pérdida de sangre y el cese del latido del corazón, en pollos de engorde aturdidos por cuatro métodos: 1) baño de agua eléctrico, 2) multifase CAS con O_2 agregado durante la fase de inducción, 3) CAS multifase sin O_2 agregado y 4) sin aturdimiento. Las aves fueron equipadas con electrodos cutáneos, (ECG) grabados con un dispositivo PhysioTel DSI, se realizó un corte de cuello en todas las aves. Los datos se analizaron mediante análisis de varianza (ANOVA), y se realizó una correlación de Pearson entre el EGG y la pérdida de sangre de acuerdo con los métodos de sacrificio. Las aves no aturdidas o aturdidas eléctricamente perdieron más sangre en comparación con las aves CAS con o sin O_2. A los 10 segundos, hubo una diferencia estadística para pérdida de sangre. La interrupción del latido cardíaco ocurrió más rápidamente para las aves no aturdidas y eléctricamente aturdidas en comparación con CAS con o sin O_2. Para las aves CAS, la aplicación del corte en el cuello no impactó el cese del latido cardíaco. El uso de CAS no alteró el cese del latido cardíaco en comparación con las aves no aturdidas o eléctricamente. Tanto los métodos tradicionales como los de CAS pueden ser utilizados en los mercados Halal.

Palabras clave: Electrocardiograma, halal, pérdida de sangre, sacrificio.

TABLE OF CONTENTS

LIST OF CHARTS, FIGURES AND APPENDICES

Charts Page

Figures Page

Appendices Page

1. INTRODUCTION

Currently, the poultry sector is one of the fastest growing industries worldwide (FAO 2019). This is because its production is carried out in short periods of time and in large quantities, all of which is supported by growth in domestic and foreign demand (USDA ERS 2018). In the US industry, Alabama ranks number two in broiler production across the country. There are a total of 18 processing plants in Alabama in which more than half of the processing industries have certifications to carry out Halal religious slaughter. Depending on the Halal certifying body, allowable stunning methods can range from no stunning at all to unrecoverable controlled atmosphere stunning (Shahdan *et al.* 2016). Until recently, waterbath stunning was the most common method of stunning poultry and low voltage waterbath is the preferred method of stunning for Halal meat production because is reversible (Fuseini *et al*. 2018). This is why entities such as the US Poultry and Egg Export Council (USPEEC) an Emirates Authority for Standardization and Metrology (ESMA) are working for the approval of more current Halal processing methods that can be carried out on a large scale, but always maintaining the parameters that this method requires.

According to Shadan *et al*. 2016, currently to carry out the slaughter method Halal has been accepted that industries use pre-slaughter methods; however, these should not interfere with:

- A swift killing by the neck cut
- The flowing of blood following the neck cutting
- Cause the animal's death (heart stoppage) prior to neck cutting
- Cause any additional stress or pain

Broiler stunning is not required in the US, but is certainly preferred. Halal does not require stunning. In fact, some customers such as Saudi Arabia do not allow stunning. For stunning, inducing unconsciousness, prior to slaughter (neck cutting) is based on the understanding that animals are sentient beings and neck cutting causes pain and suffering, which can be avoided by pre-slaughter stunning (Girasole *et al*. 2016).

One of the most common stunning methods used in the industry is the electric waterbath system. This consists of putting the broilers´ feet in the shackles, after that, the heads of the birds are immersed in salt water. If the wings are in greater contact with the water, inadequate stunning can occur. If the head is not in contact with the water this could cause the current not to pass through the central nervous system and there will be no loss of consciousness (Berg and Raj 2015).

Conventionally, a metal strip at the base of the water bath forms the positive electrode and the shackles form the negative electrode so that the electric current flows through the bird from head to legs (Berg and Raj 2015). However, methods that are more accepted by consumers such as Controlled Atmosphere Stunnig (CAS) have now emerged and increased in recent years. Gas stunning does not lead to immediate unconsciousness, but rather induces unconsciousness gradually. Current CAS systems are multi-phase and have induction, transition, and completion steps (Mckeegan *et al.* 2007). During the induction phase, birds are exposed to low levels of carbon dioxide (< 40%, hypercapnic). Addition of oxygen (hyperoxygenic) to atmospheric levels in some CAS systems is thought to minimize bird stress during this initiation phase. By the time birds reach the transition phase (40-60% carbon dioxide), they have lost posture and are considered to be unconscious. This medium level of carbon dioxide prior to high levels of carbon dioxide during the completion phase (70-85%) is thought to minimize involuntary convulsions that occur in unconscious birds during stunning. The time required from the start of the stun until unconsciousness is not instantaneous and if any birds are not fully stunned, recovery can occur very quickly. The CAS process usually requires 5-8 minutes of gas exposure with concentrations increasing from about 20 to 80% throughout the system (Berg and Raj 2015; Coenen *et al.* 2009).

Even though using CAS systems is perceived to be the better choice for animal welfare, there are several barriers against switching from electrical to CAS systems. These include high investment costs, the larger space requirement, planning requirements for gas holding tanks, inexperience with CAS systems, and the inability to slaughter for Halal markets.

Our research goal was to assess the potential for CAS systems for Halal slaughter. For this reason, the objectives for this study were the following:

- Assess physiological impact of controlled atmosphere system and waterbath stunning methods for broilers for Halal market.
- Record and analyze electrocardiograms before, during and after stunning with cessation of heart activity as an endpoint.
- Measure rate and volumes of blood loss after stunning.

2. MATERIALS AND METHODS

Location.
The project was carried out in the poultry farm of the Department of Poultry Science of the Auburn University, Alabama, United States of America. The electrical waterbath stunning process was carried out in the Poultry Research Unit – Auburn University Poultry Science Processing Plant. The controlled atmosphere stunning system was housed in an adjacent building.

Experimental design.
Four different methods of stunning, were evaluated: table 2: electrical waterbath stunning (method a), CAS without oxygen (method b), CAS with oxygen (method c) and no stunning (control group d) in broiler chickens. For this study, we used six broiler chickens for each of the treatments, with the exception of treatment d (Control group) in which only three chickens were used, thus having a total of 21 chickens per replicates, three repetitions per treatment were performed in a completely randomized desing.

Waterbath stunning method.
The first stunning method consisted in applying an electric current of low voltage (15 volts) to the head of the animal. The broiler´s legs were put in the shackle, after that, the bird´s head was immersed in a high salinity solution to increase electrical conductivity. The bird´s head passed across a metal plate to stun the animal and leave it unconscious long enough so that, it did not suffer subsequent neck cutting. For the waterbath stunning protocol, electrocardiograms could not be recorded during stunning due to electrical interference. For this method, electrocardiograms were recorded prior to and immediately following stunning at the industry standard of 15 V direct current for 10 seconds.

CAS with and without oxygen.
In this method, broiler chickens were also outfitted with a DSI PhysioTel telemetry device for the recording of electrocardiograms prior to, during, and following controlled atmosphere stunning. The hypercapnic/hyperoxygenic multi-phase CAS treatment consisted of five chambers of increasing carbon dioxide concentrations with added oxygen within chambers 1 and 2. The hypercapnic multi-phase CAS treatment was conducted using the same carbon dioxide concentrations, but without the addition of oxygen during the induction phase. Table 1 exhibits the approximate concentrations of carbon dioxide and oxygen gasses and chamber stunning

Table 1. Approximate concentration of carbon dioxide and oxygen gasses and chamber stunning.

Phase	Chamber	Carbon Dioxide (hypercapnic)	Oxygen (hyperoxygenic)	Oxygen (no added oxygen)	Time
Induction	1	20%	20%	17%	39 s
	2	25%	20%	16%	62 s
Transition	3	45%	15%	15%	67 s
Completion	4	75%	10%	10%	63 s
	5	75%	8%	8%	62 s

No stunning method.
This method was taken as a control group since it did not use any type of stunning, only a neck cut per bird was made. A DSI PhysioTel telemetry device was used for recording the electrocardiograms.

Data collection.
For each method, the birds were randomly selected, the broiler chickens were outfitted with a DSI PhysioTel telemetry device for the recording of electrocardiograms prior to, during, and following the stunning methods. For all stunning methods, heart rate was suppressed (< 180 beats per min) within 100 s following stunning. For each stunning method, volume of blood loss was recorded during exsanguination (loss of blood following neck cut leading to death). Exsanguination was conducted manually (figure 1). In order to determine if birds were dying from hypoxia or blood loss, half of birds received a neck cut within 45 seconds of exiting the CAS system while half did not. To record blood volume, the weight of blood lost over time was recorded on an individual bird basis. Broilers were placed in a cone (figure 1); this cone was secured to a balance in which chickens were weighted and the amount of blood lost by the animal was recorded every 10 seconds, for six minutes per bird. The effect of time on the percentage of chicken blood loss was also evaluated. Table 2 shows all experimental groups used in this study.

Table 2. Broiler stunning treatments.

Stunning Method Treatments	Neck Cut	
Waterbath	Yes	No
CAS without O_2	Yes	No
CAS with O_2	Yes	No
No stunning	Yes	N/A

CAS (Controlled Atmosphere Stunning).
N/A (Not Applicate).

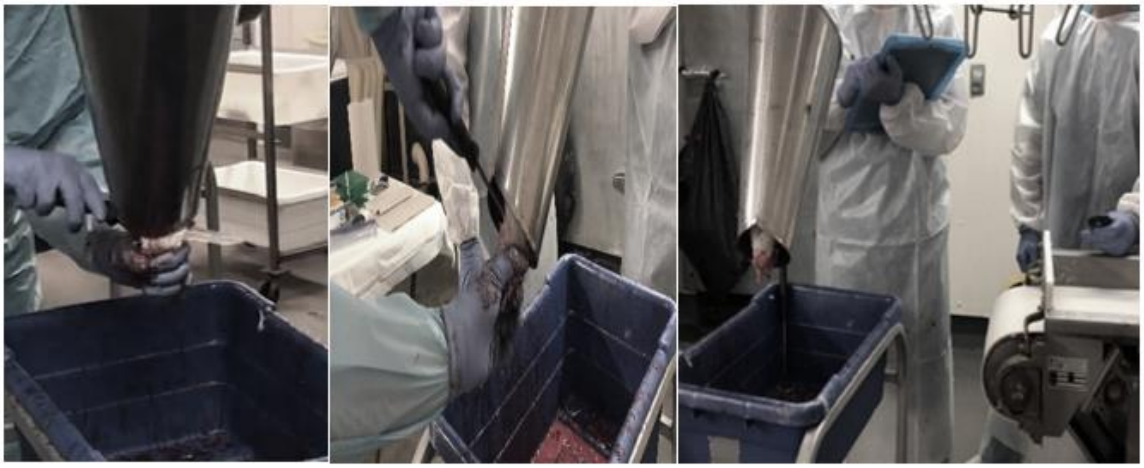

Figure 1. Broiler neck cutting and weight recordings.

Analysis of results.

First, the normality of the data was verified by the Kolmogorov Smirnov test. For the uniformity of the variance, Bartlett's test was used. Electrocardiograms for each stunning treatment method were recorded and analyzed using Ponemah software v6. 41 for both heart rate and waveform. Data were processed by analysis of variance (ANOVA) of simple classification in a completely randomized design. In the necessary cases, Duncan test (1995) was used to determine the differences among means, according to the statistical software SPSS version 17.1. LSMeans was used to measure the interaction between time and blood loss due to treatment, using SAS 9.4 program. In addition, a Pearson correlation analysis was performed between electrocardiograms and blood loss according to different methods of stunning (P value ≤ 0.01).

3. RESULTS AND DISCUSSION

Effect of stunning methods on blood loss.

According to Shadan *et al*. (2016), one important aspect of the production of Halal chicken is the method of stunning. Depending on the Halal certifying body, allowable stunning methods can range from no stunning at all to unrecoverable atmosphere stunning. For the analysis of the data, only the first minute was used and not the six minutes, since until that moment no statistical differences were found. Figure 2 shows the effect of stunning methods on blood loss. Statistically differences in the first 60 seconds ($P < 0.001$) were observed between Waterbath (15 V) and CAS with and without oxygen treatments, having higher blood loss birds stunned with Waterbath. These differences could be attributed to the lower heart rate at time of neck cut to which the animals were subjected, since before the electric stunning, the birds are removed from the chicken coops, physically manipulated by the employees of the plant while they were conscious, and hung upside down with shackles between being electrically stunned. Additionally, the electrical current (15 V) aided in the animals blood loss. Contreras and Beraquet (2001) and Craig and Fletcher (1997), indicate that stunning voltage had a significant effect on blood loss. In their study, stunned birds with lower voltages (20 to 40 volts) had higher blood losses than those stunned with higher voltages (80 to 100 volts). However, during this same study, the non-stunned birds had the lowest volume of blood loss, indicating that the electric stunning improved blood flow. Contrary, to what found in this study ($P < 0.001$).

The studies by Berg and Raj (2015), hypothesized that birds will experience pain and distress if they are exposed to carbon dioxide at high concentrations (> 40%). Birds have chemoreceptors sensitive to carbon dioxide, which induces head shakes or gasping. There are several barriers against changing electrical systems to CAS systems. These include the inability to sacrifice for Halal markets. According to Shadan *et al*. (2016), the systems used for Halal should not be subjected to such stress and the birds should be alive at the time of neck cutting. That is why the welfare argument often develops if the benefits of eliminating dump and live chaining with electrical stunning exceed the discomfort experienced during the time required to induce unconsciousness during the CAS.

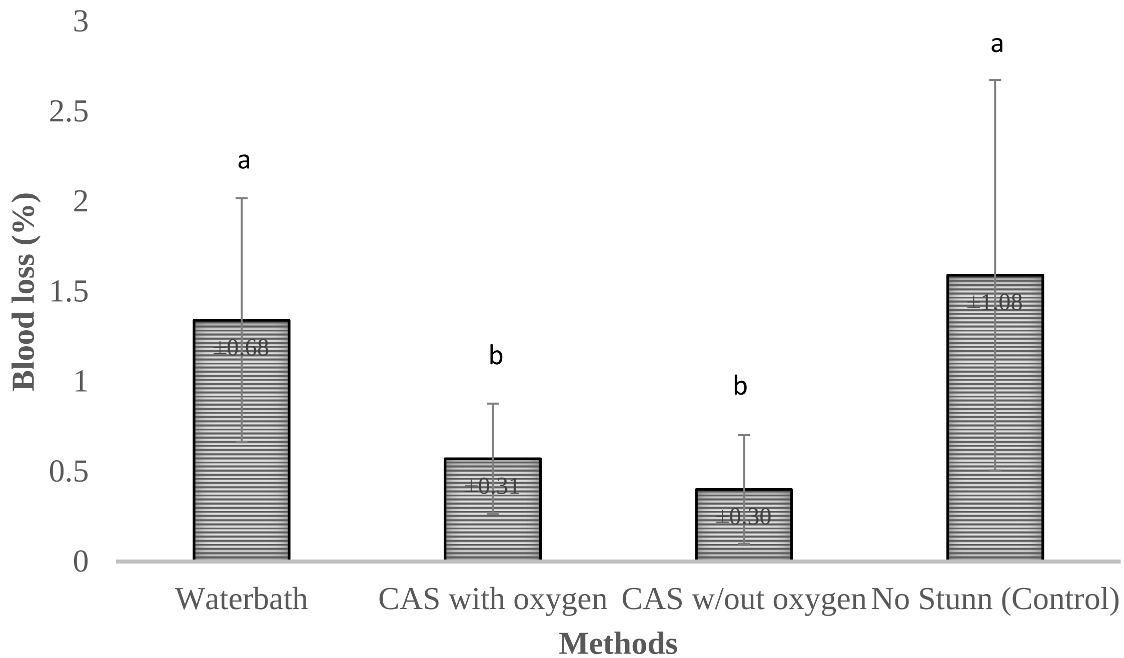

Figure 2. Effect of stunning method on percentage of blood loss in 60 seconds.
CAS Controlled Atmosphere Stunning.
(SEM± 0.073; P value <0.001).
abc Bars with different letters represent statistical difference among stunning methods ($P < 0.001$).

Other studies by Contreras and Beraquet (2001), and Craig and Fletcher (1997), indicate that stunning voltage had a significant effect on blood loss. In their study, stunned birds with lower voltages (20 to 40 volts) had higher blood losses than those stunned with higher voltages (80 to 100 volts). However, during this same study, the non-stunned birds had the lowest volume of blood loss, indicating that the electric stunning improved blood flow. CAS systems are perceived as the best option for animal welfare, there are several barriers against changing electrical systems to CAS systems. These includes the inability to slaugther for Halal markets, is due to the necessity that the birds die from blood loss, not from stunning. This separation is not clear with CAS birds because they cannot recover consciousness following stunning. At relatively low concentrations, the birds had no respiratory reflex and advanced to death with or without a cut in the neck. According to Shadan *et al*. (2016), the systems used for halal should not be subjected such stress and the birds should be alive at the time of neck cutting. These data are very similar to those found by Nakyinsige *et al.* (2014), they reported a significantly higher blood loss in rabbits with the halal slaughter method without stunning (just neck cut) than the gas stun-killing.

Effect of post neck-cutting time on broiler blood loss.

The amount of blood removed from poultry carcasses during slaughter is important (Harris and Carter 1977). In processing poultry for the market, the total and relative amount of blood lost through bleeding has always been of interest from the economic standpoint as well as concerning the appearance of the dressed poultry (Newell and Shaffner 1950). Table 3 shows the effect of post neck-cutting time on blood loss of broilers. In the times of 20, 30, 40, 50 and 60 seconds, there was no statistical difference between the waterbath and the non-stunning and also between the CAS with and without oxygen. In the time of 10 seconds there was no statistical difference between non-stunning, waterbath and CAS with and without oxygen. And it can be seen that despite no statistical differences between them, higher percentages of blood loss were obtained in non-stunning and waterbath stunning.

Table 3. Effect of after neck-cutting time on blood loss of broiler.

	Treatments ≠			
Times	Waterbath	CAS w/out O_2	CAS with O_2	No stunning
10	0.5870 ± 0.27^a	0.0875 ± 0.05^b	0.1373 ± 0.11^b	0.3301 ± 0.39^{ab}
20	0.9569 ± 0.56^a	0.2775 ± 0.13^b	0.3806 ± 0.19^b	1.1176 ± 0.74^a
30	1.3213 ± 0.59^a	0.3410 ± 0.22^b	0.5541 ± 0.13^b	1.7655 ± 0.84^a
40	1.5423 ± 0.58^a	0.5007 ± 0.24^b	0.6744 ± 0.15^b	2.0279 ± 1.16^a
50	1.7753 ± 0.49^a	0.5969 ± 0.30^b	0.7740 ± 0.18^b	2.0967 ± 0.88^a
60	1.8602 ± 0.47^a	0.5871 ± 0.38^b	0.8894 ± 0.21^b	2.2115 ± 0.92^a

(SEM± 0.140; P value <0.001).

[ab] Means with different letters in the same column represent statistical difference among in different times (P < 0.001).

[≠] No statistical different among treatments (P > 0.05)

In a study by Harris and Carter (1977), they used Kosher and decapitation, as a method of killing; in which it shown that greater blood losses were obtain by the Kosher method than by decapitation. This study also showed that between 50 and 60 seconds, birds' loss 3.8% of their blood, between 60-70 sec., they lost 2.6% and between 70 and 80 sec., they lost 1.9%. In this same study, they also used two forms of manual and mechanical cutting and found that manually slaughtered chickens lost 23.6% more blood in total bleeding time than mechanically slaughtered birds.

The results obtained in this study were different than those by Harris and Carter (1977), regarding the percentage of blood loss with over time. This because in this study, we cut everything, two carotids and two jugulars in comparison with Harris and Carter (1977), which cut only one jugular. According to Shadan *et al*. (2016), an adequate cut with a sharp tool should be made, since when an incision is made in the skin during the neck cut, the amount of vascular endothelial cells (VES) injured would be smaller compared to those if the cut it is done using a blunt tool Shadan discussed VES but in this work he obtained this information from (Inoue 1998). The number of injured VES can determine the degree of blood clotting (Chambers and Grandin, 2001), if this is done in a bad way the blood vessels are injured, causing premature coagulation and blockage of blood vessels. Therefore, it is important to cut the neck with a sharp knife, to ensure maximum and rapid loss of blood.

In processing poultry for market, the total and relative amount of blood lost through bleeding has always been of interest from the economic standpoint this is related to the efficiency of both the bleeding and the process, time is vital to save costs in the industry since there are plants that process hundreds of chickens per minute, so the shorter the time and the greater the loss of blood process is more efficient. As well as with respect to the appearance of the chickens (Newell and Shaffner 1950). Helmut (2010) stated that with all practices of slaughtering, efficient and rapid bleeding are a vital part of the slaughtering procedure. It is commonly done by severing the major blood vessels, though bleeding techniques are species-specific. Removal of blood from the carcass is essential if the meat is to be used for human consumption because it improves quality and extends the shelf life of the meat.

Effect of stunning methods on the heart beat sensation.

Shadan *et al*. (2016), declare that stunning should not cause the death of the animal due to cardiac arrest; this should be exclusive to blood loss. In religious slaughter such as Halal and Kosher, stunning should only cause the unconsciousness of the animal before the neck is cut. Table 4 shows the effect of stunning on the heart beat sensation. There was no statistical difference between the methods used and the cessation of heartbeats. This may have been due to suppression of heart rate that occurs during CAS.

Table 4. Effect of stunning methods on the heart beat cessations.

Stunning methods on blood loss of broiler					
Items	**Waterbath**	**CAS w/O_2.**	**CAS with O_2.**	**No stunning (Control)**	**R^2**
ECG (min)	12.10±3.35	12.15±4.80	12.15±3.68	10.54±2.92	0.2403

(P 0.840).
ECG (Electrocardiograms).
CAS (Controlled Atmosphere Stunning).

With the results observed in tables 3 and 4, it may be possible to suggest CAS systems (with and without Oxygen) as viable stunning methods for Halal markets. Although there was a difference between stunning methods, waterbath was no different from CAS with oxygen, and not stunning was not different from CAS without oxygen. Both, eater bath and not stunning are already approved for Halal operations.

According to Berry *et al.* (2017), carbon dioxide induces unconsciousness by reducing the pH of the blood, cerebrospinal fluid and the brain when the pH falls below the normal level induces unconsciousness. This type of stunning is sometimes not accepted by Halal or Kosher slaughter methods because at high doses of carbon dioxide the birds do not recover consciousness and may be the cause of cardiac or respiratory arrests. Additionally, birds suffer irritation in the mucous membrane and breathing difficulties, which causes stress in the animal. When the birds are electrically stunned, they become unconscious immediately after the capture of electric current, which is almost instantaneous with the difference that they can regain consciousness after a few minutes if the neck is not cut.

Several methods are used by scientist/industry people to determine unconsciousness including observation of corneal reflex response, eye blinking, limb movement, and spontaneous breathing. However, EEG (Electroencephalogram) analysis is considered the most conclusive scientific method (Coenen *et al*. 2009). Moreover, scientific investigation of the effects of stunning on brain function in various animals (broilers, turkeys, pig, and sheep) has resulted in its increased use to precisely determine (loss of) consciousness (Barbut 2015). At present, there are no investigations in which the use of EEG against ECG can be compared, because the electrocardiograms end immediately after stunning, at this point, the bird is no longer breathing but still has cardiac activity. In the controlled atmosphere stun, it is not clear if broilers die from hypoxia (lack of respiratory response) or due to blood loss before cardiac arrest.

The physiological impacts of using high levels of carbon dioxide gas during poultry stunning are relatively unknown. In many cases, previous research investigating carbon dioxide gas stunning of poultry are more than 10 years old and stunning parameter treatments are not currently in use by the poultry industry. Some previous work includes gas combinations with inert gases, such as Argon, single or bi-phasic systems, or no direct comparison with electrical stunning systems (McKeegan *et al*. 2007; Kang and Sams 1999).

Although the stunning of broilers that use a CAS system initially decreases blood loss. In their study, Mouchonie`re *et al*. (1999), the results show that when the stunning did not induce cardiac arrest, maximum blood was obtained after two minutes, while for dead animals, 90% of blood loss was obtained within this period. The maximum difference in the rate of blood loss because of inducing cardiac arrest was most evident during the first 40 s of bleeding. Figure 4 shows a normal electrocardiogram since all peaks of the heart rate are uniform.

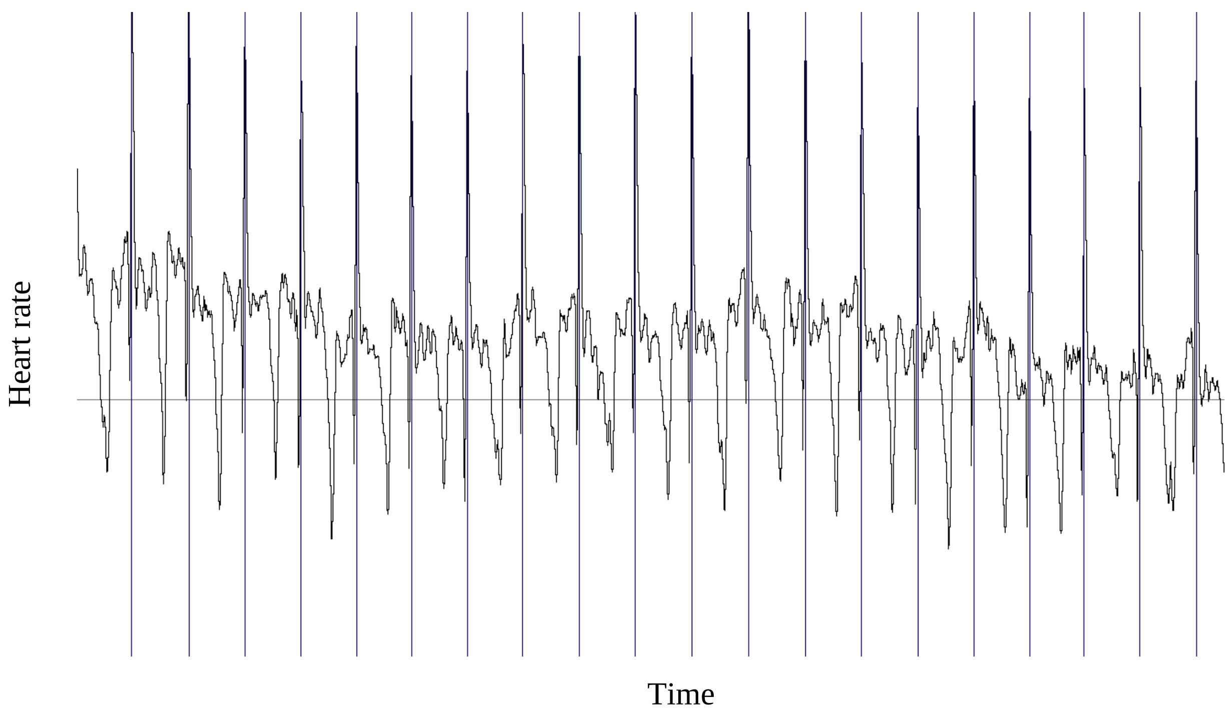

Figure 3. Normal electrocardiogram recording of heart rate for a broiler chicken.

Correlation between the ECG and blood loss of broiler.

There is a negative correlation (-0.375; significant at 0.01 level) between blood loss and the final time of death, this means that the longer the animal passed the less blood was lost. This could be due to factors such as blood and clotting time. At present there are few trials comparing these two factors, however, a study by Newell and Shafnner (1950), observed that blood clotting was not a determining factor in how the bird's body completely bled appears after of death. The amount of bleeding seems to be more closely related to the cessation of the action of the heart. They also noticed that there was little correlation between the percentage of blood loss and the appearance of the bird as regards degree of bleeding.

4. CONCLUSIONS

- The new CAS method was similar to the methods accepted by the Halal sacrifice, this means that both systems can be viable for use in Halal markets.

- The use of CAS did not alter heart beat cessation in comparison to electrically or non-stunned birds.

- The electrical and non- stunning methods was the best for blood loss.

5. RECOMMENDATIONS

- Continue with future research regarding the most appropriate stunning systems for the production of poultry and poultry products.

- Measure stress levels by means of corticosterone and the possible brain damage that different stunning methods can cause.

6. REFFERENCES

Barbut Shai. 2015. The Science of Poultry and Meat Processing. Guelph, Ontario, Canada: [publisher unknown]; [accessed 2019 Sep 19]. 764 p. http://download.poultryandmeatprocessing.com/v01/SciPoultryAndMeatProcessing%20-%20Barbut%20-%20v01.pdf.

Berg C, Raj M. 2015. A Review of Different Stunning Methods for Poultry-Animal Welfare Aspects (Stunning Methods for Poultry). Animals (Basel). 5(4):1207–1219. eng. doi:10.3390/ani5040407.

Berry W, Bourassa D, Davis J, Hess J, Johnson J, Morey A, Wallace R, 2017. Advancements in Poultry Stunning; [accessed 2019 Aug 7]. https://www.meatinstitute.org/index.php?ht=a/GetDocumentAction/i/127631.

Chambers P. G, Grandin T. 2001. Guidelines for humane handling, transport and slaughter of livestock. [place unknown]: FAO, HSI; [accessed 2019 Sep 8]. 94 p. http://www.fao.org/3/a-x6909e.pdf.

Coenen AML, Lankhaar J, Lowe JC, McKeegan DEF. 2009. Remote monitoring of electroencephalogram, electrocardiogram, and behavior during controlled atmosphere stunning in broilers: implications for welfare. Poult Sci. 88(1):10–19. eng. doi:10.3382/ps.2008-00120.

Contreras CC, Beraquet NJ. Electrical Stunning, Hot Boning, and Quality of Chicken Breast Meat. Poult Sci. 80(4):501–507.

Craig EW, Fletcher DL. 1997. A comparison of high current and low voltage electrical stunning systems on broiler breast rigor development and meat quality. Poult Sci. 76(8):1178–1181. eng. doi:10.1093/ps/76.8.1178.

Davis LL, Coe ME. 1954. Bleeding of Chickens During Killing Operations. Poult Sci. 33(3):616–619. doi:10.3382/ps.0330616.

FAO- Food Agriculture Organization of the United Nations. 2019. Production | Gateway to poultry production and products. [Accessed 2019 Feb 2]. http://www.fao.org/poultry-production products/production/en/?fbclid=IwAR2uv3vdxlFvvgqrBneD62VgjEIBjtHz4jzCTd3yOeoL0IOtY6KkglfvkZQ

Fuseini A, Teye M, Wotton S, Lines J, Knowles T. 2018. Electrical water bath stunning for Halal poultry meat production: Animal welfare issues and compatibility with the Halal rules. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources. 13(16):1–7. English. doi: 10.1079/PAVSNNR201813016.

Girasole M, Marrone R, Anastasio A, Chianese A, Mercogliano R, Cortesi ML. 2016. Effect of electrical water bath stunning on physical reflexes of broilers: evaluation of stunning efficacy under field conditions. Poult Sci. 95(5):1205–1210. doi:10.3382/ps/pew017.

Harris CE, Carter TA. 1977. Broiler Blood Losses with Manual and Mechanical Killers. Poult Sci. 56(6):1827–1831. https://academic.oup.com/ps/article-pdf/56/6/1827/4471815/poultrysci56-1827.pdf. doi:10.3382/ps.0561827.

Hemult P. 2010. Review of Stunning and Halal Slaughter. [place unknown]: [publisher unknown]; [accessed 2019 Sep 22]. 26 p. http://www.livecorp.com.au/LC/files/98/98780c7e-2e95-4e45-98d9-74ff85c50281.pdf.

Inoue, T., Y. Sakai, K. Hoshi, I. Yaguchi, T. Fujito, and S. Morooka.1998. Lower expression of neutrophil adhesion molecule indicatesless vessel wall injury and might explain lower restenosis rate aftercutting balloon angioplasty. Circulation. 97:2511–2518.

Kang IS, Sams AR. 1999. Bleedout efficiency, carcass damage, and rigor mortis development following electrical stunning or carbon dioxide stunning on a shackle line. Poult Sci. 78(1):139–143. eng. doi:10.1093/ps/78.1.139.

Livestock and Poultry: World Markets and Trade. Apps.fas.usda.gov. 2019 [accessed 2019 Feb 3]. https://apps.fas.usda.gov/psdonline/circulars/livestock_poultry.pdf?fbclid=IwAR0vMI3Yw1smtAZVtHIqEOnO3eBT3iVKfqpoaaovSHcT14pWUJeRrqY9Rnk

McKeegan DEF, SM Abeyesinghe, MA McLeman, JC Lowe, TGM Demmers, RP White, RW Kranen, H Van Bemmel, JAC Lankhaar, and CM Wathes. 2007. Controlled atmosphere stunning of broiler chickens. II. Effects on behaviour, physiology and meat quality in a commercial processing plant. Br. Poult. Sci. 48:430-442.

Mouchonière M, Le Pottier G, Fernandez X. 1999. The effect of current frequency during waterbath stunning on the physical recovery and rate and extent of bleed out in turkeys. Poult Sci. 78(3):485–489. eng. doi:10.1093/ps/78.3.485.

Nakyinsige K, Fatimah AB, Aghwan ZA, Zulkifli I, Goh YM, Sazili AQ. 2014. Bleeding Efficiency and Meat Oxidative Stability and Microbiological Quality of New Zealand White Rabbits Subjected to Halal Slaughter without Stunning and Gas Stun-killing. Asian-australas J Anim Sci. 27(3):406–413. eng. doi:10.5713/ajas.2013.13437.

Newell GW, Shaffner CS. 1950. Blood Loss by Chickens during Killing. Poult Sci. 29(2):271–275. doi:10.3382/ps.0290271.

Shahdan IA, Regenstein JM, Shahabuddin ASM, Rahman MT. 2016. Developing control points for halal slaughtering of poultry. Poult Sci. 95(7):1680–1692. eng. doi:10.3382/ps/pew092.

USDA ERS – United States Department of Agriculture Economic Research Service. Livestock and Meat International Trade Data. Ers.usda.gov. 2018 [accessed 2019 Feb 2].https://www.ers.usda.gov/data-products/livestock-and-meat-international-trade-data/livestock-and-meat-international-trade data/?fbclid=IwAR04T1K6z65Sn_cE5vt9RYhPxxl5ORuhNVXk5pA72AchDFxMIR4-tiM7LhYY

7. APPENDICES

Appendix 1. Analysis for the stunning methods and the percentage of blood loss made with the SPSS 17.1 program.

metodos	Mean	Std. Deviation	N
1.00 (Waterbath)	1.3405	.68431	54
2.00 (CAS w/out O_2)	.3985	.30985	54
3.00 (CAS with O_2)	.5683	.30473	48
4.00 (no stunning)	1.5916	1.09610	48
Total	.9685	.83660	204

Appendix 2. Duncan test for the stunning methods and the percentage of blood loss

		Subset	
metodos	N	1	2
2.00 (CAS w/out O_2)	54	.3985	
3.00 (CAS with O_2)	48	.5683	
1.00 (Waterbath)	54		1.3405
4.00 (no stunning)	48		1.5916
Sig.		.205	.062

Made with the SPSS 17.1 program.
Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .454.
a Uses Harmonic Mean Sample Size = 50.824.
b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
c Alpha = .05.

Appendix 3. Method of stunning and birds per treatment.

		N
VAR00024	1.00 (Waterbath)	13
	2.00(CAS w/out O_2)	17
	3.00(CAS with O_2)	18
	4.00(no stunning)	8

Appendix 4. Analysis of the effect of stunning methods on the heartbeat cessations with the SPSS 17.1.

VAR00024	Mean	Std. Deviation	N
1.00 (Waterbath)	12.0938	3.35074	13
2.00(CAS w/out O_2)	12.1476	4.80837	17
3.00(CAS with O_2)	12.1428	3.68137	18
4.00(no stunning)	10.5363	2.92459	8
Total	11.6534	3.83221	56

Appendix 5. Correlation between the electrocardiograms and blood loss of broiler.

Items	Blood loss (second)
ECG (min)	-0.375**

S** Correlation is significant at the 0.01 level

Appendix 6. Effect of stunning methods on blood loss of broiler.

	Stunning methods on blood loss of broiler					
Items	Waterbath	CAS w/O_2	CAS with O_2	No stunning	SEM±	P value
Blood loss (%)	1.34	0.40	0.57	1.59	0.073	<0.001

Appendix 7. Analysis of variance of the effect blood loss in time with the SPSS 17.1.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	29.548(a)	5	5.910	10.398	.000
Intercept	191.356	1	191.356	336.692	.000
segundo	29.548	5	5.910	10.398	.000
Error	112.532	198	.568		
Total	333.436	204			
Corrected Total	142.080	203			

a R Squared = .208 (Adjusted R Squared = .188)
Dependent Variable: sangre

Appendix 8. Analysis of variance of the effect blood loss with the SPSS 17.1.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	51.341(b)	3	17.114	37.720	.000
Intercept	193.140	1	193.140	425.702	.000
metodos	51.341	3	17.114	37.720	.000
Error	90.739	200	.454		
Total	333.436	204			
Corrected Total	142.080	203			

a Computed using alpha = .05
b R Squared = .361 (Adjusted R Squared = .352)

Appendix 9. Analysis of variance the ECG between blood losses with the SPSS 17.1.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	12.801(b)	3	4.267	.279	.840
Intercept	6724.906	1	6724.906	439.911	.000
VAR00024	12.801	3	4.267	.279	.840
Error	794.922	52	15.287		
Total	8412.610	56			
Corrected Total	807.723	55			

a Computed using alpha = .05
b R Squared = .016 (Adjusted R Squared = -.041)

Appendix 10. LSMEAN analysis for the time and the methods of stunning with SAS 9.4.

Tiempo	Tr	PSangre LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
10	**CASwith**	0.13731479	0.19287674	0.4774	1
10	**CASwout**	0.08746569	0.18184594	0.6311	2
10	**Nostunn**	0.33011065	0.19287674	0.0887	3
10	**WB**	0.58696746	0.18184594	0.0015	4
20	**CASwith**	0.38064283	0.19287674	0.0500	5
20	**CASwout**	0.27749472	0.18184594	0.1288	6
20	**Nostunn**	1.11760818	0.19287674	<.0001	7
20	**WB**	0.95686261	0.18184594	<.0001	8
30	**CASwith**	0.55410482	0.19287674	0.0046	9
30	**CASwout**	0.34099205	0.18184594	0.0624	10
30	**Nostunn**	1.76550488	0.19287674	<.0001	11
30	**WB**	1.32131555	0.18184594	<.0001	12
40	**CASwith**	0.67435418	0.19287674	0.0006	13
40	**CASwout**	0.50074850	0.18184594	0.0065	14
40	**Nostunn**	2.02787095	0.19287674	<.0001	15
40	**WB**	1.54232364	0.18184594	<.0001	16
50	**CASwith**	0.77399321	0.19287674	<.0001	17
50	**CASwout**	0.59693313	0.18184594	0.0012	18
50	**Nostunn**	2.09672687	0.19287674	<.0001	19
50	**WB**	1.77530142	0.18184594	<.0001	20
60	**CASwith**	0.88944397	0.19287674	<.0001	21
60	**CASwout**	0.58711432	0.18184594	0.0015	22
60	**Nostunn**	2.21152130	0.19287674	<.0001	23
60	**WB**	1.86020395	0.18184594	<.0001	24

Appendix 11. LSMEAN test for the time on the blood lost for the different stunning methods with SAS 9.4.

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	23	88.5099613	3.8482592	12.93	<.0001
Error	180	53.5700716	0.2976115		
Corrected Total	203	142.0800329			

Appendix 12. LSMEAN test for the time on the blood lost for the different stunning methods with SAS 9.4.

R-Square	**Coeff Var**	**Root MSE**	**PSangre Mean**
0.622958	56.32724	0.545538	0.968515