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Carcass Characteristics and Meat quality assessments in Broiler Chickens subject to different Pre-Slaughter Restraining Methods

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ABSTRACT

This research aimed to determine how the pre-slaughter restraint method affected broiler chickens' bleeding efficiency, carcass characteristics, and physicochemical qualities. Before slaughter, 30 male Ross broiler chickens were randomly allocated to either shackling or cone restraint. The individual blood loss of each bird was determined by comparing their body weight before and after 90 seconds of exsanguination. On the pectoralis major muscle, meat quality measures including pH, water-holding capacity, colour, tenderness, and total bacterial counts were determined. At the same time, the incidences of haemorrhage on the breast and thigh of each carcass were analysed morphologically. It was found that shackling produced less blood loss than cone restraint. Except for the final pH, shackling significantly affected the quality of the end product, as muscle lightness, shear force, drip loss, cooking loss, lipid-protein oxidation, and bacterial counts increased ($p<0.05$). A lower reflective density of myosin heavy chain was found in the muscle of broiler chickens subjected to shackle restraint compared to cone restraint. However, actin was not different between the pre-slaughter restraining techniques. In addition, broiler birds held by the shackle exhibited larger ($p<0.05$) haemorrhages than those restrained by the cone. The results indicated that the method of restraint might affect bleed-out and carcass and meat quality in broiler chickens; consequently, it should be examined in future research.

خصائص الذبيحة وجودة النوعية للحم فروج اللحم الخاضع لطرق التقيد المختلفة ما قبل الذبح

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الخلاصة

هدفت الدراسة إلى تحديد كيفية تأثير طريقة تقييد ما قبل الذبح على كفاءة نزف الدجاج اللحم وخصائص الذبيحة والصفات الفيزيائية والكيميائية للحم. قبل الذبح، تم تخصيص ثلاثون (30) من ذكور فروج اللحم عشوائياً إما للتقييد المعلق أو التقييد المخروطي. تم حساب فقدان الدم لكل طائر عن طريق فرق وزن الطائر قبل وبعد 90 ثانية من الاستنزاف. كمية الدم المتبقي بالذبيحة قدر بحساب تركيز الهيموجلوبين في العضلة الصدرية الرئيسية. شملت مقاييس جودة اللحوم للعضلة الصدرية الرئيسية الاس الهيدروجيني، القدرة على الاحتفاظ بالماء، اللون، الطراوة، أكسدة البروتين والدهن والتعداد البكتيري. بالإضافة إلى ذلك، تم تقييم النزف او الكدمات (بقع الدم) على صدر وفخذ لكل ذبيحة ظاهرياً. أشارت النتائج الافراخ الذين تم تقييدهم بالتعليق فقدوا كمية الدم أقل بكثير من أولئك الذين في التقيد مخروطي. بستثناء الاس الهيدروجيني النهائي، أثر التقيد بطريقة التعليق بشكل كبير على الصفات النوعية للحم، بما في ذلك اللون، قوة القص (الطراوة) ، قدرة على فقد الماء بالتنقيط والطهي. وفقًا لقيم المواد المتفاعلة لحمض

الثيوباربيتوريك، ادى التقييد بطريقة التعليق قبل الذبح إلى ارتفاع معنوي في قيم أكسدة دهون ليوم الاول والخامس من الخزن بالتبريد مقارنةً بالتقيد المخروطي. أظهر نتائج تحليل محتوى الكربونيل والثيول بأن طريقة تقييد قبل الذبح لم يكن لها أي تأثير على أكسدة بروتين اللحوم في اليوم الأول من الخزن بالتبريد، لكن كان محتوى الثيول والكربونيل في لحوم الطيور المقيدة بالتعليق أعلى معنوياً من محتوهما في لحوم الطيور المقيدة بالمخروط عند اليوم الخامس من الخزن بالتبريد. لوحظ انخفاض في حزم بروتين الميوسين في عضلات فروج اللحم المقيدة بالتعليق مقارنة بفروج اللحم المقيدة بالمخروط بينما كانت حزم الأكتين أكثر استقرار نسبياً بين طرق التقييد ما قبل الذبح. في اليوم الأول الخزن بالتبريد، أظهرنتائج التحليل الميكروبيولوجي للحم أن طريقة التقييد لم يؤثر معنوياً على تعداد البكتيريي بينما أظهرت عينات اللحوم التي تم جمعها من الطيور المقيدة باستخدام طريقة التعليق زيادة معنوية في تعدلد الكلي للبكتيريا مقارنة بعينات اللحوم المأخوذة من الطيور المقيدة بطريقة المخروط. الإضافة إلى ذلك ، كان لدى طيور التي تم تثبيتها بواسطة التعليق بقع دموية على الذبيحة أكثر معنوياً من تلك المقيدة بواسطة المخروط. أوضحت نتائج الدراسة الحالية بان طريقة التقييد قبل الذبح قد تؤثرعلى درجة النزف، خصائص الذبيحة والجودة النوعية للحم لدجاج اللحم، لذا يجب اجراء دراسات المستقبلية بهذا الخصوص.

الكلمات المفتاحية: التقيد المخروطى، بقع دموية على الذبيحة، جودة اللحم، فروج اللحم، التعليق

INTRODUCTION

Since prehistoric times, poultry meat, namely broiler chicken, has been an essential part of the human diet (Sabow and Majeed, 2019). Its high biologically valuable nutritional content and processing utilisation positioned it among the most popular foods (Parpia *et al.*, 2018). Additionally, Broiler chicken meat meets the needs of modern consumers due to its low fat, mostly saturated fatty acid, and cholesterol content compared to equivalent cuts of red meat such as mutton and beef (Wideman *et al.*, 2016). Despite its low lipid content, broiler chicken flesh contains many unsaturated fatty acids and is a source of conjugated linoleic acid, which has anti-inflammatory, anti-thrombotic, and atherosclerosis-preventing properties (Mir *et al.*, 2017; Moussa *et al.*, 2019). Broiler meat also has high biological value protein constituting all essential amino acids that cover human requirements. In addition, the broiler is an essential source of vitamins, especially vitamin A, E, and D, and minerals such as potassium, sodium, calcium, magnesium, iron, copper, zinc, and manganese (Angelovičová *et al.*, 2016).

Modern customers place a premium on meat quality and safety at the time of sale, influenced by genetic and environmental factors (Bostami *et al.*, 2021). Among the environmental factors are occurrences that occur just before slaughter that is known to be stressful for broiler chickens and may substantially impact the meat quality of the birds (Saraiva *et al.*, 2020). For instance, it has been observed that pre-slaughter restraining measures influence the pre-slaughter and post-slaughter physiological responses in broiler chickens, particularly energy metabolism inside the skeletal muscle, which influences post-mortem muscle metabolism. While shackling is the most prevalent method of restraining broilers in commercial settings, it has been recognised that it negatively affects broilers' welfare and meat quality (Vinco *et al.*, 2016; Fuseini *et al.*, 2018). Struggling or wing flapping during pre-slaughter shackling has been reported to fasten the initial rate of pH drop, which is often associated with poor meat quality features such as undesirable colour and poorer water-holding capacity (Huang *et al.*, 2018). Likewise, pre-slaughter conditions may cause stress and physical damage, which significantly influences lipid-protein oxidation in meat and meat products during the early post-slaughter period (Bostami *et al.*, 2021). Limiting this behaviour in shackled broilers prior to slaughter may help to create meat with optimal final pH, juiciness, and lowers the occurrence of pale, soft, and exudative meat and protein degradation, thereby boosting production, profitability, and meat quality (Huang *et al.*, 2018). In recent years, cone restraint has been discovered to limit the mobility of birds prior to slaughter, during slaughter, and shortly after slaughter. An earlier

study found that confining birds in a cone constraint and then slaughtering them reduced fowl struggle and improved meat quality compared to shackling (Ismail *et al.*, 2019; Ismail *et al.*, 2016).

However, there is a lack of detailed information on carcass defects, such as the incidence of haemorrhage, which is the primary concern in the poultry meat industry because it is one of the negative carcass characteristics that affect consumer acceptance of meat and meat quality in terms of physicochemical attributes and microbiological quality of broiler chickens during refrigerated storage. Thus, this study was conducted to determine the effects of pre-slaughter shackling and cone restraining methods on broiler chickens' bleeding efficiency, carcass, and meat quality.

MATERIALS AND METHODS

Birds and experimental design

Thirty 42-day-old male Ross broiler chickens reared under similar management system with an average live body weight of 1.925 ± 0.008 kg were purchased from a commercial poultry farm. The broiler chickens were transported from the farm to a commercial poultry abattoir (Erbil's slaughterhouse for poultry- Kurdistan Region, Iraq). They were slaughtered 60 min after arrival (Ismail *et al.*, 2019). Following transportation and release, the live bodyweight of each bird was measured and recorded. Using either a shackle or a cone, broiler birds were restrained for 30 seconds before slaughter and humanely slaughtered according to the standard halal slaughtering method. A licensed slaughter-man carried out the slaughtering procedure. Each bird's head was pulled dorsally to stretch its neck and facilitate exsanguination. Using a sharp knife, a transverse section was severed. The neck cut severed skin, muscle, oesophagus, trachea, carotid arteries, jugular veins, and major nerves to drain excess blood from the carcass without decapitating the head.

Determination of blood loss

Individual blood loss during the 90 seconds of exsanguination was measured by changing body weight before and after slaughtering (Kranen *et al.*, 1996). Calculate the percentages of blood loss using the formula provided below:

$$\text{Blood loss (\%)} = [(W1-W2)/W1] \times 100$$

Where,

W1= live weight

W2= weight after neck cut

Carcass sampling and storage

Following evisceration and carcass dressing, 20 g of pectoralis major muscle from the left side was taken, labelled, vacuum-packed, and stored at 4 ºC for drip loss assessment. The breast muscles of the dorsal side of the pectoralis major were removed from the chilled carcass and divided into two portions. The first portion (right pectoralis major muscle) was labeled correctly, vacuum packaged, and stored at -20 ºC for subsequent determination of pH, colour, cooking loss, shear force, lipid-protein oxidation, myofibrillar degradation, and microbial enumeration at d 1, whereas the second portion (left pectoralis major muscle) were vacuum packed and directly stored at 4 ºC chiller for 5 days. Upon completion of the aging period, the left pectoralis major muscle was labelled, vacuum packaged, and stored at -20 ºC until subsequent analyses.

Carcass evaluation

Using a modified version of the Bostami *et al.,* (2021) method, we observed characteristics of the corpse, such as haemorrhages, 1 day after post-mortem. The breast and thigh muscle haemorrhages were quantified using a visual grading system. The classification was performed independently by three observers knowledgeable in the carcass and meat quality. A threshold model consisting of a discontinuous 5-point scale with 4 cut-off points was used for classification. Cut-off points were formed by photographs of breast and thigh muscles showing a particular severity of haemorrhages; class 1: haemorrhage free; class 5: numerous and severe haemorrhages (Lambooij *et al.,* 1999). The severity of red wingtips on the carcass was also estimated visually using a number scale with 0 (no defects), 1 (moderate redness), and 2 (severe redness) (McNeal *et al.,* 2003)

Determination of meat quality characteristics

***Glycogen content* :** As outlined in the instruction manual, total muscle glycogen concentration was measured using the colorimetric assay EnzyChrom™ Glycogen Assay (Elabscience, USA).

***Muscle pH*:** The pH of the pectoralis major muscle was evaluated carefully and indirectly with a portable pH meter (EZDO PP-203, Taiwan). 1 gram of beef was homogenised in 20 milliliters of ice-cold deionized water for 30 seconds. Using a pH meter pre-calibrated at pH 4.0 and 7.0, the pH of the homogenates was used

***Water holding capacity*:** The meat samples' water holding capacity (WHC) measured cooking and dripped loss following procedure desicribed by Sabow (2020). Individual fresh flesh samples from the pectoralis major muscle were weighed (about 20 g) and recorded as the initial weight for drip loss (W1). The samples were placed in polyethylene plastic bags, properly labeled, vacuum sealed, and stored at 4 degrees Celsius for five days. At the prescribed storage duration, samples were removed from the polyethylene plastic bags, blotted dry, weighed, and recorded asW2. The percentage of drip loss was computed using the following formula:

Drip loss (%) = [(W1-W2) ÷ W1] × 100).

Pectoralis major muscle samples were weighed (W1), packaged in polyethylene bags, and vacuum-sealed to quantify the cooking loss. The samples were cooked in a preheated water bath (HAAKE C10, UK) set to 80 ºC for 10 minutes after reaching an internal temperature of 78 ºC, as measured with a piercing temperature probe. After removing the cooked samples from the water bath and allowing them to cool to room temperature, they were carefully dried and reweighed (W2). The proportion of cooking loss was computed using the following formula:

Cooking loss (%) = [(W1- W2) ÷ W1] × 100

***Shear force*:** The meat samples used to determine cooking loss were prepared to evaluate the shear force values using the Volodkovitch bite jaw attached to a Brookfield Texture Analyzers (CT3™, USA). The equipment was calibrated at a 10 mm return distance for height, and the blade speed was set at 10 mm/s. Samples were prepared according to Sazili *et al.,* (2005) method. Parallel to the direction of the muscle fibers, 1 cm (height) × 1 cm (width) × 2 cm (length) blocks were cut from each sample. Each block was sheared with the Volodkevitch bite jaw in the center and perpendicular to the fibers' longitudinal orientation. Measurements of shear force were recorded in kilogram (kg) units as the average peak positive force of all subsample values for each sample.

***Meat colour*:** A Colour Flex spectrophotometer was employed to assess the meat's colour (Shenzhen 3nh Technology Co., Ltd, China). Before use, the colorimeter was calibrated against black and white tiles. Blooming was applied for 30 minutes to 12 mm thick samples of the pectoralis major muscle (AMSA, 2012). The flower sample was placed on the facing base of the colorimeter cup. Each sample's L* (lightness), a* (redness), b* (yellowness), c* (chroma), and h* (hue angle) values were measured and averaged in triplicate

***Determination of thiobarbituric acid reactive substances (TBARS)*:** According to Aminzade *et al.,* (2012) and modified by Sabow *et al.,* (2020), the TBARS of the pectoralis major muscle was determined. 5 grams of pectoralis major muscle were homogenised in 48 millilitres of sterile water and 1.25 millilitres of 4N HCl for 2 minutes. The liquid was distilled to a volume of 25 ml. The distillate and the TBA reagent were boiled for 35 minutes (15% trichloroacetic acid, 0.375% thiobarbituric acid). After 10 minutes of chilling under running water, a spectrophotometer measured absorbance at 538 nm against a blank (Spectronic Instruments, USA). Increasing the optical density by 7.843 yielded the TBARS results. The oxidation products were measured in malondialdehyde equivalents (mg MDA per kilogram of meat).

***Determination of protein oxidation* :** Meat protein oxidation is evaluated by measuring the amount of protein thiols (the sulfhydryl group (SH) of a cysteine residue) and carbonyl group (Sabow *et al.,* 2016). The free thiol content and carbonyl groups were estimated using a colorimetric assay kit (ABCAM, USA) following the manufacturer's instructions. The final results were expressed in nanomoles of free thiol or carbonyl content per milligram of protein.

***Immunoblot analysis of myofibrillar proteins*:** Myofibril proteins were extracted according to Morzel *et al.,* (2006) with minor modifications (Sabow *et al.,* 2016). Briefly, 2.5 g of pectoralis major muscle were homogenised for 30 s in 20 ml of extraction buffer containing 150 mM NaCl, 25 mM KCl, 3 mM MgCl2, 4 mM EDTA at pH 6.5 to which protease inhibitor (Sigma, Germany) had been added. The homogenate was filtered to eliminate any remaining collagen. After filtration, the homogenate was incubated at 4 ºC with shaking and centrifuged at 2000 g for 15 min at 4 ºC. The pellet was washed twice with 25 ml of a 50 mM KCl solution at pH 6.4 and once with 25 ml of 20 mM phosphate buffer at pH 6. The pellet was finally re-suspended in the same phosphate buffer and stored at -20 ºC until analysis.

***Extraction of myofibrillar proteins* :** Using a protein test kit (Parsazmoon, Iran) and the colorimetric analytical technique, the protein content of the final suspension was measured (Bradford, 1976). Bovine serum albumin (BSA) was used to create protein measuring standards. Bovine serum albumen was diluted into six protein standard concentrations of 0.0, 0.1, 0.2, 0.3, 0.4, 0.5 µg/µl using the same extraction buffer which was used in the protein extraction. In duplicate, a volume of 10 µl each of sample and standard were suspended into each well of 96-well plate followed by the addition of 200 µl of Bradford dye reagent then the plate was incubated for 5 minutes at room temperature. Using a microplate reader (Awareness Stat Fax 2100, USA), the absorbance was measured at 595 nm wavelength. The standard curve was plotted and the protein concentration in each sample was determined using the equation derived from the standard curve.

***SDS-PAGE gel electrophoresis* :** Myofibrillar proteins were mixed with sample buffer containing 30% (v/v) glycerol, 5% (v/v) mercaptoethanol, 2.3% (w/v) SDS, 62.5 mM Tris–HCl (pH 6.8) and 0.05% (w/v) bromophenol blue in a 1: 1 ratio and incubated at 90 ºC for 10 min. One dimensional

SDS-PAGE was performed using polyacrylamide gels of 8 cm x 5.5cm (length x width) and 0.8 mm thickness. For actin, 12% resolving gel solution was prepared by mixing 3.4 ml deionized water, 4.0 ml of 30% degassed acrylamide/bis, 2.5 ml of 1.5 M Tris-HCl (pH 8.8) and 0.1 ml of 10% (w/v) sodium dedocyl sulphate (SDS), 50 µl of 10% (w/v) ammonium persulphate (APS) and 5 µl of tetramethylethylenediamine (TEMED). For myosin heavy chain, 5% resolve gels were prepared by mixing 5.7 ml deionised water and 1.7 ml of 30% degassed acrylamide/bis in the above mixture. The gel was overlayed with n-butanol purposely to remove any air bubbles. Whenever three layers were formed, the top two layers were poured away, and the stacking gel was poured on top of the resolving gel. The 4% stacking gel solution was prepared by mixing 6.1 ml deionized water, 1.3 ml of 30% degassed acrylamide/bis, 2.5 ml of 1.5 M Tris-HCl (pH 6.8), 0.1 ml of 10% w/v SDS, 50 µl of 10% (w/v) APS and 10 µl of TEMED. The gel was left at room temperature for 1 h to polymerise. Keeping the comb in, the gels were well wrapped in moist paper towels (to prevent drying out) and kept overnight at 4 ºC. A volume of 5 µl protein ladder (Page RulerTm Prestained Protein Ladder Plus; Cat No: SM 1811 from Fermentas Life Sciences, Canada) was loaded into the first well, while an equivalent of 20 µg proteins of each sample was loaded into the remaining wells. Proteins were separated in a running buffer containing 0.025 M Tris base, 0.192 M glycine, and 0.1 SDS at pH 8.3 under constant voltage of 120 V and 400 mA for 90 min, in which the tracking dye reached the bottom of the gel. The gels were stained with 0.05% coomassie blue staining solution for 60 minutes and destained with a destaining solution for 30 minutes. The bands of myofibrillar proteins were visualised using GS-800 Calibrated Imaging Densitometer (BIORAD, USA) (Figure 1).

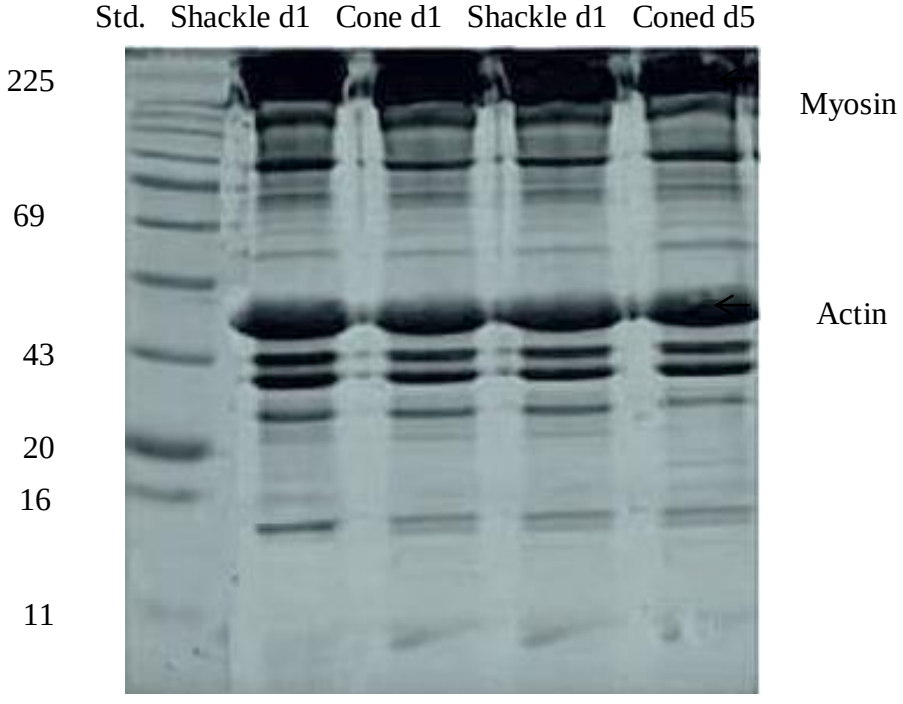


Figure 1: Representative SDS-PAGE showing the myofibrillar protein bands of pectoralis major muscle during post-mortem aging periods in poultry chickens subjected to different pre-slaughter restraining methods

Microbiological quality **:** On every sampling day (1 and 5), 1 g of meat samples from pectoralis major muscle were aseptically weighed, transferred to a plastic centrifuge tube containing 9 ml of deionized water, and homogenized for 120 s at room temperature. For microbial enumeration, 100 µl

samples of 10-fold dilution in deionized water were spread on the surface of dry media. Tenfold dilution was spread on Petri dishes in duplicate for enumerations of total aerobic count (TAC) on Plate Count Agar (NEOGEN-NCM0033A, UK). The plates were incubated at 32 ºC for 24 h. After the incubation period, all bacteria colonies (cfu/g) on TCB plates were counted and then converted to log10 CFU/g prior to statistical analysis.

Statistical Analysis

The experimental design was a completely randomized design (CRD). The General Linear Models (GLM) technique of the Statistical Analysis System (SAS, 2007) software programme, version 9.1, was utilized for statistical analysis. The results were analysed using the ANOVA procedure, with the restraining method (shackle and cone) and storage (day 1 and day 5) as the main effects and their interaction (restraining method storage) as the interaction. Duncan's multiple range test was employed to compare means when significant effects were identified. The statistical significance level was ($p<0.05$).

RESULTS and DISCUSSION

The fundamental objective of the meat processing industry is to increase blood loss during slaughter, as better blood loss can improve meat quality during storage. Blood loss in broiler chicks subjected to various pre-slaughter restraint procedures is presented in Figure 2. The restraint method significantly ($p<0.05$) affected blood loss as restraining the birds using the cone restraint resulted in higher blood loss than restraining the birds using a shackle restraint. The average blood loss during shackle restrain was 3.025 %, while the loss following cone restrain was 3.682 %. The consumption of blood is forbidden. Therefore, religious and modern slaughter requires that animals slaughtered for food be properly bled before consumption (Nakyinsige *et al.,* 2014). Numerous researches on restraining measures on blood loss have produced contradictory findings. Our findings are congruent with Ismail *et al.,* (2019), who discovered that the pre-slaughter restraint method substantially affects broiler chicken blood loss. According to the author (Ismail *et al.,* 2019), birds with cone restraints lost more blood (3.26 percent) than birds with shackles (2.97 percent). In contrast, Lambooij *et al.,* (1999) examined the effect of two post-slaughter restraining devices, the shackle, and the cone, on bleeding efficiency in broiler chickens. Blood loss relative to body weight was significantly higher in shackled birds than cone birds. They utilized light broiler chicken (about 1.5 kg live body weight) with a bleed-out rate of around 2.68 and 2.46 percent after stunning while chained or restrained, followed by neck cutting. Although hanging may promote rapid bleeding by prompting birds in a shackle line to straighten up and flap their wings, it is established that wing-flapping occurs only for a few seconds immediately following shackling. Despite this, many birds continue to flap their wings if they are unexpectedly exposed to sunlight, startled, or receive electric shocks in the bath (Ismail *et al.,* 2019).

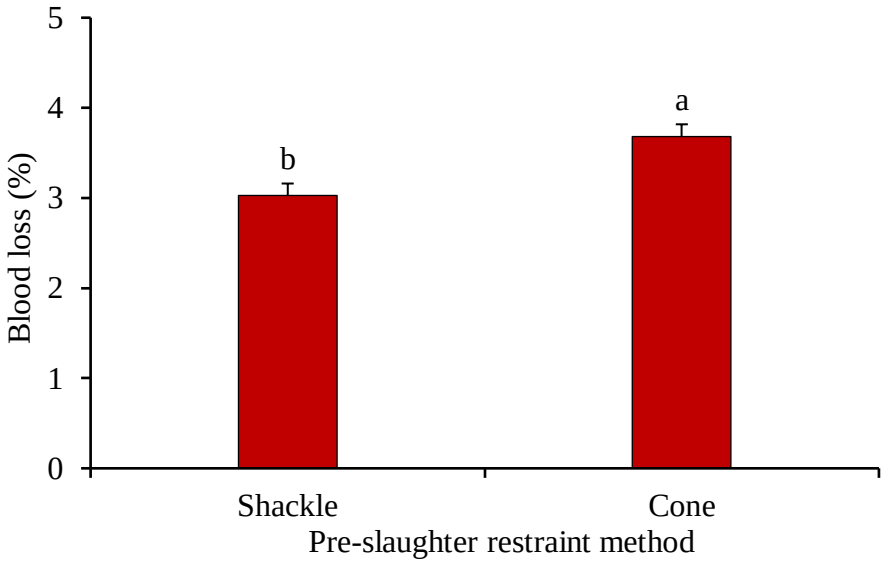
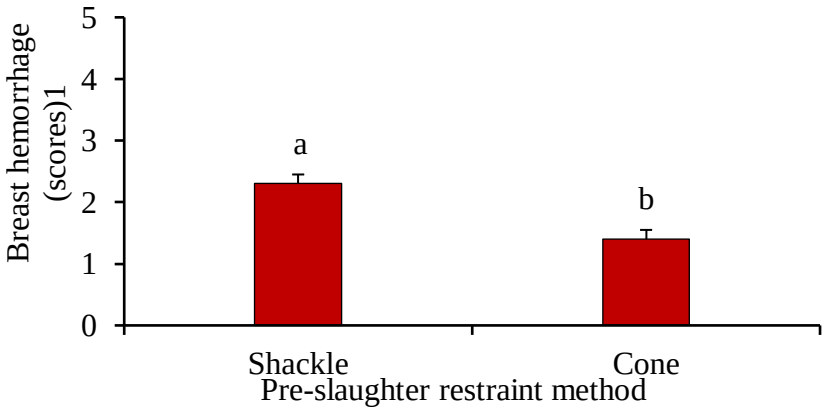


Figure 2: Blood loss as affected by pre-slaughter restraint method in broiler chickens

[a, b]Means with different letters differ significantly at $p<0.05$.
Values are mean ± standard error.

Carcass quality

Several problems with carcass quality can be directly attributed to pre-slaughter bird handling, including the restraining method. In Figure 3, details of the comparison of the carcass quality scored in terms of breast hemorrhage, thigh hemorrhage, and red wing tips between different restraining methods.



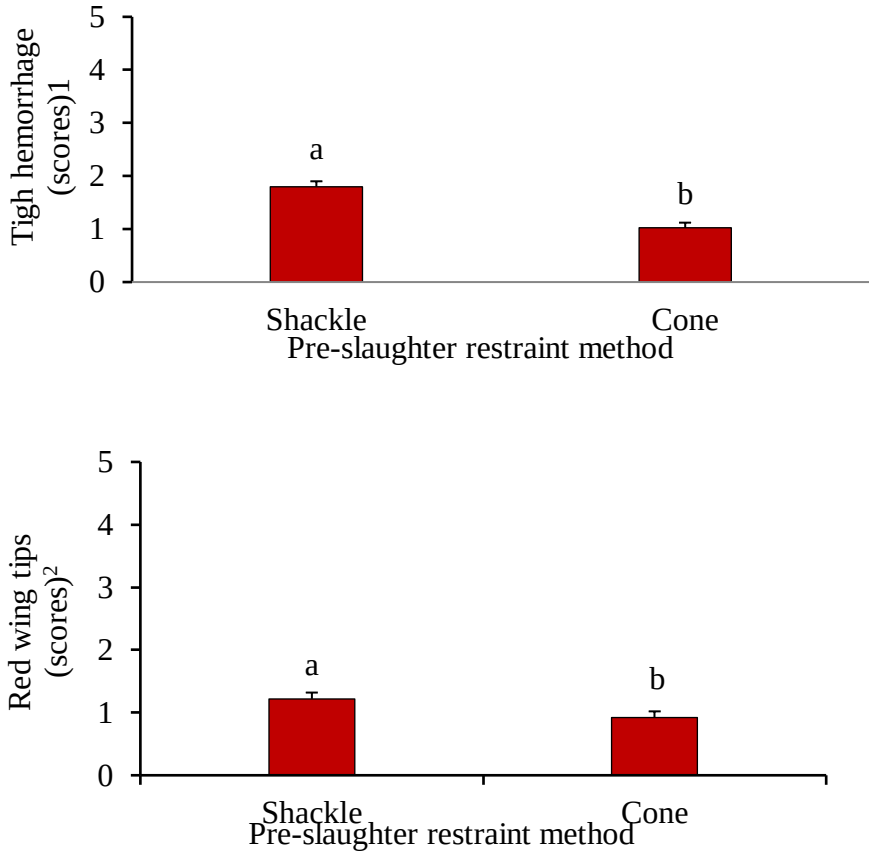


Figure 3: Carcass characteristics as affected by pre-slaughter restraint method in broiler chickens

[a, b]Means with different letters differ significantly at $p<0.05$. [1]The five-point grading scale is 1 score, free hemorrhage, to a 5 score which indicates numerous and severe hemorrhages (Lambooij *et al.*, 1999) [2]The three-point grading scale is 0 score, no red wing tips, to 2 score which indicates severe redness (McNeal *et al.*, 2003). Values are mean ± standard.

Cone restrain birds had a lower ($p<0.05$) incidence of hemorrhage in the breast, thigh, and red wingtips than those from the shackle. No incidence of broken bones was observed in both slaughter restrain groups. Hemorrhages found in broiler carcasses could be due to struggling or wing-flapping (an escape or discomfort behavior) that occurred during shackling restraint which may result in the occurrence of blood circulation disturbances as well as capillary rupture (hemorrhage). This explanation is supported by Cockram *et al.*, (2020). They stated that hemorrhages occur due to blood leakage from ruptured capillary vessels caused by flooding of capillaries due to vasodilation of arterioles following vasoconstriction stimulated by sudden muscular contraction accompanying shackling prior to slaughtering. In poultry, Lambooij *et al.*, (1999) compared cone restraining and shackling methods on carcass quality of broiler chickens. The authors found that thigh muscle hemorrhaging was higher in shackled birds than those restrained in cones. Similarly, Kittelsen *et al.*, (2015) found a significant increase in wing fractures after shackling. Ismail *et al.*, (2019) also observed that the shackled form of pre-slaughter restraining could lead to hemorrhages in the muscles and skin covering the muscle.

Meat quality

Muscle glycogen content and pH

The amount of muscle glycogen at slaughter plays a vital role in postmortem energy metabolism. Glycogen content is a reliable indicator of ante-mortem stress. Muscle glycogen content at the time of slaughter is one of the most influential factors of ultimate pH. When glycogen reserves are low at the time of slaughter, a small amount of lactic acid is formed during rigor development

resulting in high ultimate pH (Nakyinsige *et al.*, 2014). Table 1 shows the effect of different pre-slaughter restraining methods on broiler chickens' glycogen concentration of the pectoralis major muscle. At 1 and 5 d postmortem, the breast muscle from broiler chickens subjected to glycogen content did not differ ($p>0.05$) between the slaughter methods. However, at 12 h postmortem, the LL muscle from goats subjected to pre-slaughter cone restraining treatment had higher ($p<0.05$) glycogen content compared with those subjected to shackle restraining treatment. The movement of the birds increased the utilisation of adenosine triphosphate (ATP) by the muscle tissues, which may have contributed to the lower glycogen concentration in the shackle restraining group compared to the cone restraining group. The increase in the rate of glycogen metabolism has been previously attributed to the effect of behavioral reactions in birds that are hung upside down in shackles before neck cutting (Ismail *et al.*, 2019).

The amount and rate of glycogen breakdown and lactate release in the muscle pre- and post-slaughter of broiler chicken are influenced by events before slaughter handling. Table 1 presents the average pH of the pectoralis major muscle of broiler chickens subjected to various restraint methods. Although restraining methods did not affect ultimate pH ($p>0.05$), the shackle group exhibited lower muscular pH readings than the cone group. The lower value of final pH in shackled birds could be due to struggle or free wing flapping during shackling, which raises lactate concentration in breast muscle, reducing muscle pH. Ismail *et al.*, (2019) and Lambooij *et al.*, (1999) discovered comparable muscle pH in shackle- and cone-restrained broiler chickens. Regardless of treatment, the pH levels steadily rose over time. The accumulation and proteolytic cleavage of metabolites due to bacterial action on meat may account for the increase in pH during preservation (Kim *et al.*, 2019). Similar findings were made while chilling chicken breast meat (Kim *et al.*, 2020).

Table 1: Muscle glycogen and pH as affected by pre-slaughter restraint method in broiler chickens of broilers

Parameter	Storage (day)	Pre-slaughter restraint method		P value	
		Shackle	Cone	RM	RM×S
Glycogen (mg/g meat)	1	$5.246\pm 0.04b^{x}$	7.120 ± 0.01^{ax}	<.0001	0.6671
	5	4.890 ± 0.03^{by}	6.940 ± 0.01^{ay}	<.0001	
	P value	<.0001	<.0001		
pH (unit)	1	5.514 ± 0.04^{y}	5.657 ± 0.05^{y}	0.8894	0.5495
	5	5.808 ± 0.08^{x}	5.821 ± 0.02^{x}	0.5508	
	P value	<.0001	<.0001		

^{a,b} Means within the same row with different letters are significantly different at $p<0.05$.
^{x,y} Means within the same column with different superscripts are significantly different at $p<0.05$.
Values are mean ± standard error.

Water holding capacity and shear force

Table 2 depicts the Water holding capacity in drip and cooking losses of chickens subjected to different pre-slaughter restraint methods. On the first postmortem day, there was a significant ($p<0.05$) increase in drip and cooking loss among birds subjected to shackle restraint compared to cone restraint. Increased water retention in broiler chicks may be attributable to shackling-induced lactate production in the muscles. Additionally, a lower meat pH promotes protein denaturation and reduces the meat's ability to retain water (Ismail *et al.*, 2016; Huang *et al.*, 2014). These results were compared with Ismail *et al.*, (2019) and (2016), who observed that pre-slaughter shackling caused significantly greater drip and cooking loss than birds held in cones. After seven (7) days of aging, the

restraining method did not influence drip and cooking loss ($p>0.05$). Water holding capacity is the ability of meat to keep both inherent and added water when subjected to external forces such as cutting, heating, grinding, and pressing. It is a vital quality parameter in the chicken meat market since it affects palatability and economic attributes (Kaboshio *et al.,* 2020). Regardless of the treatment group, Water holding capacity increased ($p<0.05$) over time. This result may be explained by the aging-related degradation of collagens and myofibrillar proteins, which reduces the capacity of myofibrillar proteins to store water (Soglia *et al.,* 2018).

From a sensory perspective, tenderness is the most important quality characteristic determining customer acceptance of meat (Li *et al.,* 2021). The principal effects of restraint method and age on the shear force of broiler chicken meat (Table 2). Shear force values of pre-slaughter shackle restraints were significantly greater than those of cone restraints (1.133 kg vs. 1.133 kg at d 1 and 1.078 kg vs. 1.034 kg at d 7). This could be attributable to the higher water loss of meat from the shackle group during cooking. Ismail *et al.,* (2019) gave a similar explanation, which attributed the higher force of meat from the shackled chickens compared to the coned chickens to significantly higher cooking loss. Moreover, wing restraint treatment stretches the breast muscles and prevents contraction, resulting in longer sarcomeres, thus improving the tenderness of the meat. The values of shear force reduced significantly with aging. The reduction in shear force values could be due to rigor resolution caused by the enzymatic breakdown of collagen holding muscle fibers under refrigerated storage (Oliveira *et al.,* 2021). It has been reported that the final meat tenderness depends on postmortem changes during aging, which affect the contractile system of the muscle or myofibrils (Shi *et al.,* 2021; Bhat *et al.,* 2018).

Table 2: Water holding capacity and shear force as affected by pre-slaughter restraint method in broiler chickens of broilers

Parameter	Storage (day)	Pre-slaughter restraint method		P value	
		Shackle	Cone	RM	RM×S
Drip loss (%)	1	2.958 ± 0.15^{ay}	2.267 ± 0.13^{by}	0.0039	0.3713
	5	3.713 ± 0.22^{x}	3.419 ± 0.36^{x}	0.0891	
	P value	<.0001	<.0001		
Cooking loss (%)	1	29.879 ± 0.53^{ay}	27.46 ± 0.43^{bx}	0.0025	0.1996
	5	26.449 ± 0.71^{y}	24.33 ± 0.77^{y}	0.0599	
	P value	<.0001	<.0001		
Shear force (kg)	1	1.133 ± 0.02^{ax}	1.084 ± 0.01^{bx}	0.0492	0.1121
	5	1.078 ± 0.01^{ay}	1.034 ± 0.01^{ay}	0.0055	
	P value	0.0012	0.0024		

[a,b] Means within the same row with different letters are significantly different at $p<0.05$.
[x,y] Means within the same column with different superscripts are significantly different at $p<0.05$.
Values are mean ± standard error.

Colour values

The meat's colour is a valuable indicator of its freshness at the time of purchase and is one of the most influential factors in determining customer acceptability (Wang *et al.,* 2021; Hughes *et al.,* 2020). Table 3 displays the color parameters of chicken pectoralis major muscle treated with various pre-slaughter restraint techniques. Restraining methods affected meat lightness, redness, and yellowness. At one (1) day postmortem, meat from birds confined using the shackle method had significantly ($p<0.05$) higher lightness (L*) and lower redness (a*) and yellowness (b*) values than

meat from birds confined using the cone method. This can be attributable to numerically differences in pH values across various restraint methods. Salwani *et al.,* (2016) found that the colour of breast meat is frequently related to postmortem muscle pH change. According to the author (Salwani *et al.,* 2016), breast muscles with a lower pH appear less red than those with a higher pH in broiler chickens. Saláková *et al.,* (2009) observed a significant positive correlation between redness and yellowness. Mir *et al.,* (2017) posited that the redness and yellowness of meat are linked, where meat with a higher a* tends to have higher levels of b*. The restraint method did not affect ($p>0.05$) on the parameter of colour, which consists of lightness, redness, and yellowness on 5 d postmortem. In agreement with the present findings, Ismail *et al.,* (2016) also found a significant effect of the restraint method (shackle and cone) on the color coordinates (L*, a*, and b*) of broiler chickens. Although C* (Chroma) and h* (hue) values up to day 5 did not differ significantly between restraint methods, meat from birds restrained in the cone had lower chroma and hue color tone than meat from the birds restrained using the shackle method. Irrespective of the restraint method, lightness values increased ($p<0.05$), while the redness and yellowness values decreased ($p<0.05$) with increasing postmortem aging. A vital decrease in color characteristics could be due to myoglobin oxidation during aging, as it is the major hem protein responsible for meat colour (AMSA, 2012).

Table 3: Colour characteristics as affected by pre-slaughter restraint method in broiler chickens of broilers

Parameter	Storage (day)	Pre-slaughter restraint method		P value	
		Shackle	Cone	RM	RM×S
Lightness (L*)	1	62.55 ± 0.74^{ay}	60.09 ± 1.26^{by}	0.0117	0.5283
	5	63.71 ± 1.06^{x}	60.97 ± 0.91^{x}	0.1662	
	P value	0.0039	0. 0443		
Redness (a*)	1	6.102 ± 0.15^{ax}	5.51 ± 0.18^{bx}	0.0261	0.8915
	5	5.855 ± 0.20^{y}	5.47 ± 5.47^{y}	0.2295	
	P value	0.0211	0. 0224		
Yellowness (b*)	1	8.698 ± 0.28^{ax}	7.074 ± 0.22^{bx}	0.0003	0. 7431
	5	8.151 ± 0.24^{y}	7.821 ± 0.29^{y}	0.0882	
	P value	0.0307	0.0111		
Chroma (C*)	1	10.36 ± 0.12^{x}	9.910 ± 0.28^{x}	0.0670	0.6586
	5	9.986 ± 0.35^{y}	9.088 ± 0.20^{y}	0.3943	
		0.0022	0.0273		
Hue (h*)	1	48.83 ± 0.62^{y}	47.68 ± 0.80^{y}	0.2698	0.9661
	5	49.94 ± 1.08^{x}	48.89 ± 1.05^{x}	0.4990	
	P value	<.0001	<.0001		

^{a,b} Means within the same row with different letters are significantly different at $p<0.05$.
^{x,y} Means within the same column with different superscripts are significantly different at $p<0.05$.
Values are mean ± standard error.

Meat lipid-protein oxidation

Lipid oxidation is the major non-microbial cause of spoilage in meat and meat products, primarily under pro-oxidative conditions such as storage (Sabow and Majeed, 2019). Malondialdehyde (MDA) is one of the essential aldehydes produced during the secondary lipid oxidation of polyunsaturated fatty acids. It is considered the major marker of lipid oxidation. Thus, the thiobarbituric acid reactive substances (TBARS) test for malondialdehyde determination is the most commonly used method for assessing lipid oxidation in muscle because of its sensitivity and

relatively simple procedure (Domínguez *et al.*, 2019). Table 4 shows the TBARS value of muscle from broiler chickens subjected to different pre-slaughter restraining methods. At 1 and 5 d postmortems, pre-slaughter shackle restraint resulted in significantly higher lipid oxidation value than cone restrain. These values were consistent with the results for residual blood, which increases the concentration of home proteins (mainly haemoglobin) in meat. Hemoglobin is an influential promoter of lipid oxidation (Sabow *et al.*, 2016). Additionally, pre-slaughter conditions may cause stress and physical damage, greatly influencing lipid oxidation in meat and meat products during the early post-slaughter period (Bostami *et al.*, 2021). In general, lipid oxidation increased significantly over storage in both groups. However, no group (shackle or cone) had an MDA value that reached detectable concentration for humans, as established by Abdulla *et al.*, (2018). Lipid oxidation changes lead to off-odors, off-taste, discoloration, protein degradation, toxic compound accumulation, and shelf life decline, affecting consumers' health (Sabow *et al.*, 2016).

The oxidation of protein is one of the most novel challenges in meat quality evaluation throughout processing and storage because muscle tissue has a large concentration of proteins, which have an essential role in meat quality in terms of sensory, nutritional, and physicochemical aspects (Falowo *et al.*, 2014). The principal oxidative changes of protein occur on the amino acid side chains and include the production of carbonyl groups, thiol oxidation, and aromatic hydroxylation (Morzel *et al.*, 2006). As a result, the quantity of thiols and carbonyl groups in meat is commonly used to assess protein oxidation (Guyon *et al.*, 2016). Table 4 shows the results of the influence of pre-slaughter restraint measures on protein oxidation in terms of thiol and carbonyl levels in chicken. The pre-slaughter restricting approach did not influence protein oxidation values in broiler chicken flesh on day one (1) postmortem. Nonetheless, at 5 d postmortem, meat from birds restrained using the shackle method was significantly ($p<0.05$) higher in the thiol and carbonyl content than meat from birds restrained using the cone. This observation coincides with the results obtained for lipid oxidation which showed that birds restrained using a shackle had greater TBARS value than those restrained with a cone. According to Falowo *et al.*, (2014), protein oxidation occurs due to the interaction between proteins; especially the nitrogen or sulfur centers of reactive amino acid residues of protein and lipid hydro-peroxide or secondary lipid oxidation products such as aldehydes. It was also observed that the onset of lipid oxidation in meat and meat products seems to occur faster than the oxidative degradation of myofibrillar proteins. Thus, lipid-derived radicals and hydroperoxides are more likely to promote protein oxidation (Thanatsang *et al.*, 2020; Domínguez *et al.*, 2019). The free thiol content decreased, and carbonyl content increased ($p<0.05$) over storage regardless of the pre-slaughter restraining method. These observations are consistent with those of Ferreira *et al.*, (2018) and Smet *et al.*, (2008), who showed that free thiol and carbonyl groups significantly decreased and increased as meat ages.

Table 4: Lipid-protein oxidation of meat as affected by pre-slaughter restraint method in broiler chickens of broilers

Parameter	Storage (day)	Pre-slaughter restraint method		P value	
		Shackle	Cone	RM	RM×S
Lipid oxidation (mg MDA/kg meat)	1	0.669 ± 0.01^{ay}	0.424 ± 0.01^{by}	0.0214	0.330
	5	2.475 ± 0.01^{ax}	1.931 ± 0.01^{bx}	<.0001	
	P value	<.0001	<.0001		
Free thiol (nmole/mg protein)	1	48.24 ± 0.19^{x}	49.74 ± 0.29^{x}	0.198	0.302
	5	41.37 ± 0.38^{by}	46.01 ± 0.73^{ay}	<.0001	
	P value	<.0001	<.0001		
Carbonyl (nmole/mg protein)	1	2.320 ± 0.01^{y}	2.210 ± 0.007^{y}	0.622	0.347
	5	2.976 ± 0.01^{ax}	2.523 ± 0.015^{bx}	0.011	
	P value	<.0001	<.0001		

[a,b] Means within the same row with different letters are significantly different at $p<0.05$.
[x,y] Means within the same column with different superscripts are significantly different at $p<0.05$.
Values are mean ± standard error.

Myofibrillar Protein Profile (myosin and actin)

The intensities of myosin heavy chain and actin proteins were quantified by measuring each detected band's reflective density (RD). The effects of the restraint method on the degradation of myosin heavy chain and actin of muscle during postmortem aging are shown in Table 5. Generally, the restraining method had an effect ($p<0.05$) on myosin heavy chain values. A lower reflective density of myosin heavy chain was found in the muscle of broiler chickens subjected to shackle restraint compared to cone restraint. However, the reflective density of actin was not significantly different between the pre-slaughter restraining techniques. The higher degradation of myosin protein in the shackle group compared with the cone group could be due to protein oxidation as demonstrated through loss of thiol groups and increased carbonyl concentrations. An increase in oxidation can enhance protein degradation by proteases (Xue *et al.,* 2012). According to Nakyinsige *et al.,* (2015), protein oxidation enhances the degradation of myosin heavy chain and actin. Myosin is the most abundant protein in the myofibril complex, and it comprises about 45% of the total myofibrillar proteins in the muscle tissues of birds (Nieto *et al.,* 2013). Ooizumi and Xiong (2004) indicated that the initial oxidation of chicken myofibrils induced changes in myosin, particularly intermolecular cross-linking of myosin heavy chain and modifications of thiol groups at the myosin ATPase active site. Myofibrillar proteins are particularly susceptible to oxidative reactions, with myosin being the most sensitive (Domínguez *et al.,* 2022). It has been reported that actin protein plays a significant role in muscle contraction (Xue *et al.,* 2012). The present research showed that actin was not affected by both pre-slaughter restraining methods (Table 5). This oxidative stability of actin may be attributable to the inaccessibility of oxidation sites, in which myofibrillar suspensions may be masked by the interaction of actin with myosin chains (Xue *et al.,* 2012). Nakyinsige *et al.,* (2015) in rabbits found that the degradation of actin is minimal during refrigerated storage. Elsewhere, actin bands have also been found to be relatively stable even under oxidative conditions of μ-calpain (Xue *et al.,* 2012) and chemical-induced oxidation (Morzel *et al.,* 2006).

Table 5: The degradation of myosin and actin (reflective density/mm2) of muscle as affected by pre-slaughter restraint method in broiler chickens of broilers

Parameter	Storage (day)	Pre-slaughter restraint method		P-value	
		Shackle	Cone	RM	RM×S
Myosin	1	56.930 ± 0.65^{bx}	58.713 ± 0.36^{ax}	<.0001	0.566
	5	53.153 ± 0.31^{by}	57.987 ± 0.93^{ay}	<.0001	
	P value	<.0001	<.0001		
Actin	1	18.263 ± 0.09	18.874 ± 0.34	0.144	0.719
	5	18.102 ± 0.02	18.786 ± 0.37	0.227	
	P value	0.634	0.533		

[a,b] Means within the same row with different letters are significantly different at $p<0.05$.
[x,y] Means within the same column with different superscripts are significantly different at $p<0.05$.
Values are mean ± standard error.

Microbiological analyses

Figure 4 shows the effect of the pre-slaughter restraining method on microbial levels of breast meat from broilers during the first five days postmortem. At d 1, microbial counts were not significantly different for the two pre-slaughter restraining methods. However, at d 5 postmortem, more significant growth of total bacterial counts was indicated by meat samples obtained from the birds restrained using the shackle method than the meat samples obtained from birds restrained in the cone ($p<0.05$). The higher bacterial growth exhibited by broiler chickens subjected to shackle restraint can be attributable to the low blood loss. According to Bourbab and Idaomar (2012), residual blood in the carcass after bleeding is one of the most significant factors affecting contamination. It is a perfect medium for microorganisms' growth due to its high nutritive value. Some authors have also demonstrated that meat from broiler chickens that have suffered stress during the process of slaughtering is likely to have a shorter shelf-life due to spoilage since the glycogen levels in the muscles are not enough to develop the maximum level of lactic acid and an ideal pH (Iannetti *et al.,* 2020; Petracci *et al.,* 2010). In general, increased growth of microorganisms with storage time was detected in both pre-slaughter restraining groups and meat samples from the shackled chickens with the highest counts of total bacteria. However, the levels of total bacteria counts in both pre-slaughter restraining groups were within the acceptable limits. Rouger *et al.,* (2017) reported that spoilage of poultry meat occurs when total bacterial counts reach 6 - 7 log cfu/g.

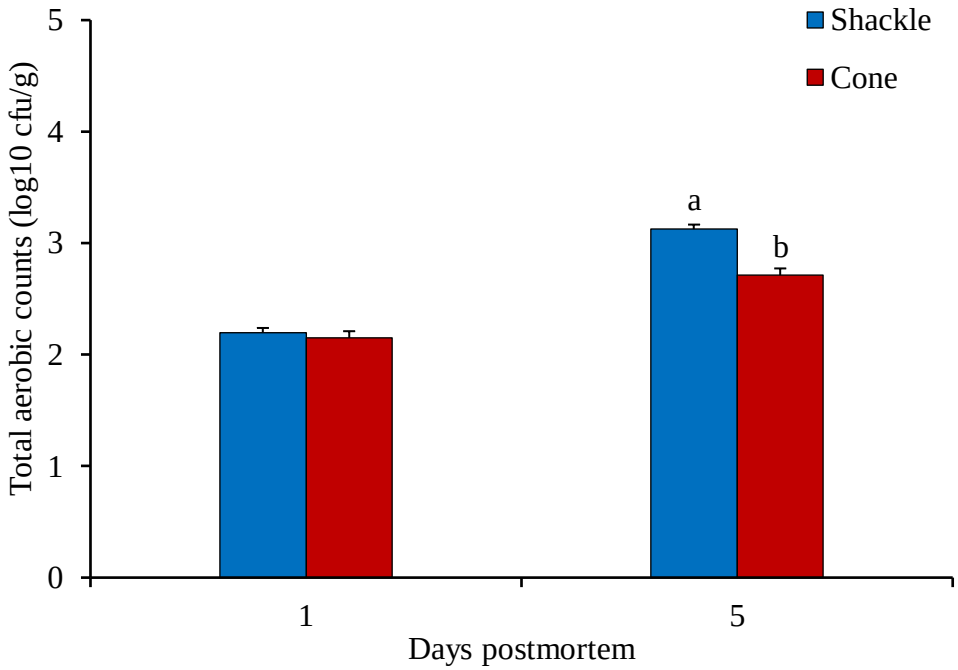


Figure 4: Total bacteria counts in chicken breast muscle as affected by pre-slaughter restraint method in broiler chickens

[a, b]Means with different letters differ significantly at $p<0.05$.
Values are mean ± standard error.

CONCLUSION

The present study results indicate that, except for ultimate pH, the pre-slaughter restraint method significantly affected meat quality characteristics such as color, water holding capacity, and tenderness. Due to the limited bleed-out, the shackle method had a detrimental effect on lipid-protein oxidation and overall bacterial counts in broiler chicken meat. There was a higher incidence of breast and thigh hemorrhage in the shackle group than in the cone group. Consequently, the cone can be substituted for conventional restraint methods to produce broiler chicken meat with increased meat quality and shelf life following postmortem aging.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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