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EFFECTS OF SHACKLING AND CONE RESTRAINING ON RESIDUAL BLOOD IN CARCASS AND PHYSIOLOGICAL STRESS RESPONSES OF BROILER CHICKENS

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Abstract

The effects of restraint methods on stress hormones (corticosterone, adrenaline, and noradrenaline), blood biochemistry (glucose and lactate), and bleeding efficiency were examined in broiler chickens. Thirty 30 male Ross broiler birds were randomly allocated to either shackling or cone restraint prior to the neck cut. As measures of well-being, physiological data such as alterations in blood metabolites and hormone levels were assessed. Each bird's blood was calculated by comparing its initial body before and after the neck cut for 90 s. The amount of leftover blood was determined using the haemoglobin content of the *Pectoralis major* muscle. Compared to their shackled counterparts, the plasma levels of adrenaline, noradrenaline, glucose, and lactate were lower in the coned broiler chickens ($p<0.05$). Those shackled lost significantly less blood than those restrained in a cone. Coned broilers showed significantly less residual haemoglobin in their breast muscles than shackled broilers. It can be concluded that pre-slaughter cone restraint is preferable to shackle restraint. However, a feasible restraint device has not yet been developed.

Keywords: Broiler, Pre-slaughter restraining, Residual blood, Shackling, Welfare.

تأثير التقييد المعلق والمخروطي على الدم المتبقي في الذبيحة واستجابات الإجهاد الفسيولوجي لدجاج اللحم

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الملخص

تم دراسة تأثير طرق التقيد على هرمونات الإجهاد (الكورتيكوستيرون والأدرينالين والنورادرينالين)، والكيمياء الحيوية للدم (الجلوكوز واللاكتيك) وكفاءة النزف في الفروج اللحم. تم تخصيص ثلاثين 30 ذكور من فروج اللحم عشوائياً إما للتقييد المعلق أو التقييد المخروطي قبل الذبح. قبل يوم واحد من النقل. كمقاييس للرفاه، تم تقييم البيانات الفسيولوجية مثل التغيرات في نواتج الايض ومستويات الهرمون في الدم. تم حساب كمية دم المفقودة من كل طائر من خلال فرق وزن الجسم قبل وبعد الذبح بمدة 90 ثانية. تم تحديد كمية الدم المتبقية بالذبيحة بقياس محتوى الهيموجلوبين في العضلة الصدرية الرئيسية. بالمقارنة مع نظرائهم المقيدة، كانت مستويات البلازما من الأدرينالين والنورادرينالين والجلوكوز واللاكتات أقل معنويا في الدجاج اللحم بالتقيد المخروطي. الافراغ المقيدين بالتعلق فقدوا دمًا اقل بشكل معنوي من أولئك المقيدين بالتقيد المخروطي. أظهرت الافراغ المقيدة بالتقيد المخروطي انخفاضًا ملحوظًا في نسبة الهيموجلوبين المتبقي في عضلة الصدرية مقارنة بالفروج المقيدة بالتعليق. يمكن الاستنتاج بان التقييد المخروطي قبل الذبح أفضل من التقييد بالتعليق. ومع ذلك، لم يتم تطوير أداة تقييد مناسبة لحد الان.

كلمات مفتاحية: فروج اللحم، التقيد قبل الذبح، الدم المتبقي، التعليق، الرفق بالحيوان.

Introduction

The poultry industry is currently one of the fastest-growing industries globally due to its capacity to generate vast quantities of products in a short length of time, which is supported by rising local and worldwide demand. In addition, poultry meat, notably broiler chickens, satisfies the modern demand for low-fat diets (6). Contrastingly to other-meat producing animals, the birds are hung upside down in shackles before neck cutting. Pre-slaughter shackling is unavoidable in commercial slaughter plants (10). Stress due to pre-slaughter handling has a significant impact on their well-being and is a significant cause of economic loss in the poultry industry. Also (1) reported that pre-slaughter transport of birds negatively affects meat yield and meat quality. Nevertheless, shortly before the neck cut, climatic factors have been considered distressing for broiler birds and may adversely affect meat quality. For example, the

pre-slaughter restraint method was reported to have noticeable effects on post-slaughter physiological responses in broiler chickens (3). These changes could alter protein content in muscle, reflecting many biochemical processes (2). According to (3), cone restraint has been found to limit the movement of birds before neck cut, during neck cut, and shortly after neck cutting. The study observed that birds' flailing of the wings and struggling during pre-slaughter shackling, the most common method of holding live birds in a commercial setting, has been detrimental to their welfare. In addition, a poor bleed-out in broiler chickens that occurred during shackling restraint can also be observed by hemorrhagic conditions of the meat (3). Blood loss is a critical concern for meat processors, as residual blood in the carcass is often related to decreased shelf life. Thus, optimizing blood loss at slaughter to ensure product quality, promote shelf and reduce meat and carcass defects is a primary concern of the meat processing industry (7).

Even though there is evidence of the effects of shackling and cone constraint on broiler chicks, little to no data on the effect of shackling and cone restraint on broiler bird welfare and residual blood from carcasses is scarce. The most recent study on the impact of shackling and cone restraint on stress hormones in broiler hens is an exception (3). These variables necessitate a study to investigate the influence of limitations on broiler chickens, which are becoming increasingly popular due to high demand and welfare problems associated with pre-slaughter handling. As a result, the current study examined the effects of shackling and cone restraining on blood biochemistry (glucose and lactate), stress-related hormones (corticosterone, adrenaline, noradrenaline), and residual blood from broiler birds carcasses as welfare indicators.

Materials and Methods

Thirty 30 42-day-old male Ross broiler chickens, with an average live body weight of 1.925±0.008 kg were purchased from a commercial poultry farm. Transport of the birds was carried out from the commercial farm to the lairage pens of the abattoir (Erbil's chicken slaughter plant in Kurdistan Region, Iraq). The birds were individually weighed and recorded as their live weight after transportation and lairage. One hour after their arrival, the birds were slaughtered (3). Immediately before slaughter, the birds were subjected to shackle or cone restraint for 30 s shown in (Figure 1) below and humanely slaughtered following the traditional Halal slaughter technique. A licensed slaughter-man carried out the slaughtering procedure. Individually, the heads of the birds were pulled dorsally to stretch the neck to facilitate exsanguination. A transverse section was performed with a sharp knife. The neck cut severed skin, muscle, oesophagus, trachea, carotid arteries, jugular, and major nerves without decapitating the head or draining excess blood from the carcass.

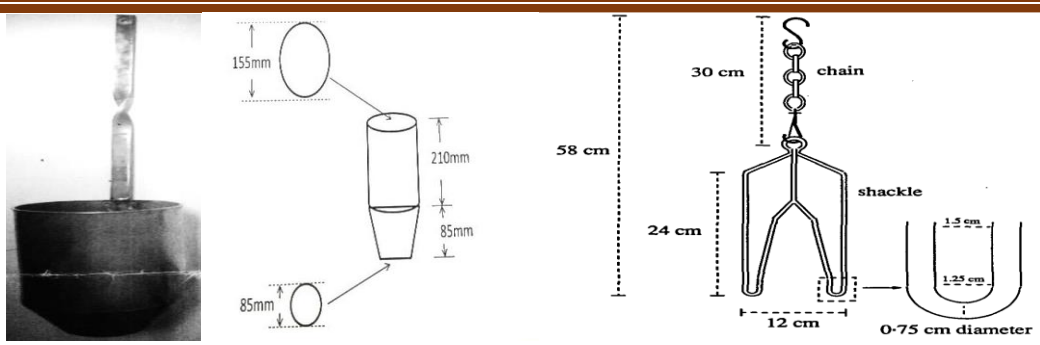

Figure 1 The design and dimensions of shackles and cone restrainer.

Blood Sampling, five mL of sticky blood was obtained through jugular venipuncture during slaughter and put in blood tubes containing EDTA as an anticoagulant (BD Franklin Lakes, NJ, USA) and preserved in an icebox. The blood samples were centrifuged for 15 minutes at 3000 x g 4^{o}C, and the extracted plasma section was kept at -20^{o}C until further analysis. After exsanguination for 90 seconds, the slaughtered birds were individually weighed, and their weights were recorded as the weight after slaughter.

Determination of Physiological Stress, Commercial kits (ZellBio GmbH, Germany) were used to assess glucose and lactic acid concentrations. The analyses were carried out for each variable according to the manufacturer's instructions.

Determination of Stress-linked Hormones, Bird hormone levels were determined using an ELISA kit (ZellBio GmbH, Germany). Adrenaline and noradrenaline levels were tested concurrently using the appropriate ELISA High Sensitive kits (MyBioSource, USA). The samples were measured in triplicates, as directed by the manufacturer.

Determination of Blood Loss, to determine each bird's blood loss during the exsanguination's 90s, researchers compared the bird's weight before and after it was slaughtered (4). The following calculation was used to compute the amount of blood lost:

Blood loss (percentage) = [(W1-W2)/W1] × 100

W1 is the live weight.

W2 is the weight after the neck has been severed.

Quantification of Haemoglobin Protein, the muscle haemoglobin measured using colorimetric assay kit (abcam, USA). After dressing the carcass and chilling at 4 ºC for 24hr, 5g of muscle sample from Pectoralis major was added to added to 15 mL of ice-cold extraction buffer (80mM KCl, Tris-HCL, pH 8) and homogenised for 40 s. Twenty microliter of each diluted muscle sample or standard (different concentrations 0-50 mg/mL) were pipetted into a 96 well micro-plate and then 180 µl of hemoglobin detector added, thoroughly mixed, and allowed to stand at room temperature for 15 minutes. The absorbance was measured at 575 nm with a micro-plate reader (awareness Stat fax 2100, USA). The standard curve was plotted and the haemoglobin concentration in each sample was calculated using the equation derived from the standard curve.

Statistical analysis, the experiment was set up in a completely random design (CRD). The General Linear Models (GLM) method of the Statistical Analysis System (SAS) programme version 9.3 was used for statistical analysis. Duncan's multiple range tests was employed to compare the means when significant effects were found. The statistical significance level was set to ($p<0.05$).

Results and Discussion

Many studies have used biochemical blood parameters such as glucose and lactate as indicators of stress in poultry because of their ability to respond to a wide range of stressors (11). The effect of the restraint technique on plasma glucose and lactate concentrations is displayed in Table 1. In this investigation, the values of biochemical blood parameters taken from birds confined in various ways prior to slaughter varied considerably. The group restrained with shackles had higher blood glucose and lactic acid levels than those restrained with cones ($p<0.05$). The increased plasma glucose and lactate levels could be attributed to struggle or wing flapping during shackling. This finding is comparable with (3), who discovered a substantial difference in broiler chickens' blood glucose and lactate levels after shackle and cone restraint.

Table 1 Plasma metabolites as affected by the pre-slaughter restraint method in broiler chickens.

Parameter	Pre-slaughter Restraint Method		P-value
	Shackle	Cone	
Glucose (mmole/l)	13.47 ± 0.05^{a}	12.87 ± 0.10^{b}	0.0003
Lactate (mmole/l)	3.932 ± 0.02^{a}	2.577 ± 0.02^{b}	0.0011

^{a,b} at $p<0.05$, the means with different letters differ significantly

Values are mean ± standard error.

Table 2 displays the effects of the restraint technique on plasma corticosterone, adrenaline, and noradrenaline concentrations. Generally, plasma corticosterone values were not influenced ($p< 0.05$) by the pre-slaughter restraining technique. However, the restraint technique substantially affected adrenaline and noradrenaline levels, with birds restrained with a shackle having higher levels than those restrained with a cone. Accordingly, stress involves an immediate (2 minutes) response by catecholamine neurotransmitters (e.g., adrenaline and noradrenaline) and a medium-term (20 minutes) response by non-genomic and subsequently the genomic effects of corticosteroid hormones (e.g., corticosterone in birds). Also, at the early stage of the stress response, both catecholamines and corticosteroids are elevated, while at a later stage of the stress response, only the corticosteroids remain elevated (8). This may explain why the shackling method in the current experiment only influenced adrenaline and noradrenaline but not corticosterone. With high-energy conditions, substantial quantities of adrenaline and noradrenaline are released into the bloodstream to aid body organisation. Consequently, shackling may induce stress in chickens by stimulating the sympathetic adrenal medullary neural system, releasing noradrenaline and adrenaline from sympathetic endings and the adrenal medulla. A study by (3) discovered that the shacking group of birds had more significant amounts

of stress-related compounds than the cone group. Also (2) found that chickens kept in chains had greater levels of stress chemicals than those that had been bled.

Table 2 Stress-linked hormones as affected by pre-slaughter restraint method in broiler chickens.

Parameter	Pre-slaughter restraint method		P-value
	Shackle	Cone	
Corticosterone (ng/ml)	1.867 ± 0.02	1.827 ± 0.01	0.082
Adrenaline (ng/ml)	2.215 ± 0.02[a]	1.295 ± 0.01[b]	0.001
Noradrenaline (ng/ml)	2.817 ± 0.03[a]	2.045 ± 0.02[b]	0.014

[a,b] At p<0.05, the means with different letters differ significantly.
Values are mean ± standard error.

The principal objective of the meat processing industry is to increase blood loss following slaughter, as greater blood loss correlates with superior meat quality throughout storage. Figure 2 depicts blood loss in broiler chicks treated to various pre-slaughter restraint measures. The type of restraint had a statistically significant (p<0.05) impact on blood loss, with cone constraint resulting in greater bloating. Blood loss was significantly (p<0.05) influenced by the method of restraint, with cone restraint resulting in greater blood loss than shackles. Instead of shackling, there is blood loss. It is prohibited to consume blood. Therefore, religious and modern slaughter standards stipulate that animals slaughtered for human food must be wholly bled before consumption (7). Multiple studies by (7) and (10) on blood loss mitigation techniques have yielded inconclusive results. Our findings are congruent with (3) and (5), who found that pre-slaughter restraint substantially impacts broiler chicken blood loss. The author found that birds with cone restraints lost more blood than those with shackles (3). Although it is often believed that shackling causes birds in a shackle line to stand upright and flap their wings, research has revealed that this behavior occurs for only a few seconds after shackling. Many birds flail their wings when scared, exposed to sunshine, or shocked by electricity (3).

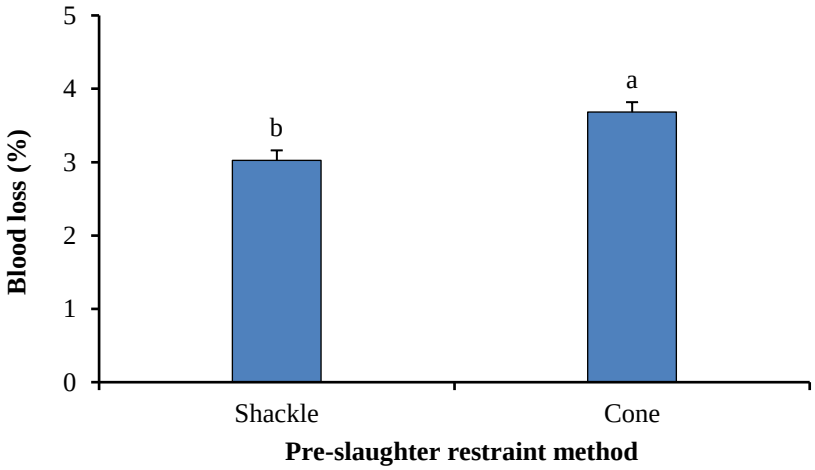

Figure 2 Blood loss as affected by pre-slaughter restraint method in broiler chickens.

[a,b] At p<0.05, the means with different letters differ significantly.

Values are mean ± standard error.

Figure 3 displays the concentrations of residual haemoglobin in the Pectoralis major muscle of hens restrained in various ways. Cone restraint produced a lower residual haemoglobin content than shackle restraint ($p<0.05$). This discovery may be related to blood loss during the two pre-slaughter restraint methods. Due to the high haemoglobin content, beef is susceptible to lipid oxidation. Haemoglobin accelerates lipid oxidation via various mechanisms, such as pseudo-lipoxygenase-like activities, which can shorten the shelf life of meat and meat products (19).

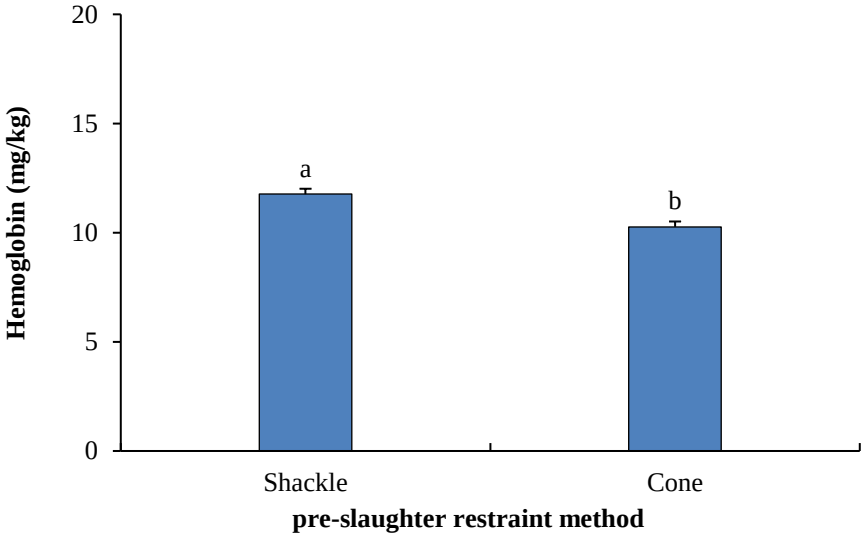

Figure 3 Residual haemoglobin content in chicken breast muscle as affected by pre-slaughter restraint method in broiler chickens.

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

In conclusion, the results revealed that the restraint method influenced broiler chickens' stress reactivity and bleeding efficiency. Cone restraining is resulted in less blood loss and residual haemoglobin in the muscle than shackling. In addition, compared to shackled broilers, cone-restricted broilers showed reduced plasma levels of glucose, lactate, adrenaline, and noradrenaline. Cone restraint with slaughter provides advantages over shackle restraint; however, additional study is necessary to achieve a satisfactory restraint.

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