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Original Article

# Evaluation of Quality and Safety Attributes of Slaughtered Versus Dead Chicken Birds Meat

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## ABSTRACT

The adulteration of dead chicken meat with halal meat is a concern in Pakistan that can harm safety of meat as well as religious beliefs of the Muslims. Accordingly, the present study was conducted to evaluate slaughtering methods (Islamic and decapitation) with dead chicken meat on composition, quality and safety attributes. Purposely, (n=24) birds were slaughtered and (n=12) dead birds samples were collected and subjected to proximate, mineral and quality analysis including pH, color (L*, a*, b*), cooking loss, texture, as well as thiobarbituric acid reactive substances (TBARS), peroxide value (PV), haem and non-haem iron. The results indicated ash content, minerals and oxidation parameters including TBARS, PV affected significantly ($p$<0.05). The highest Iron (Fe) and Magnesium (Mg) levels reported in dead bird meat were 14.21±0.99 and 959.62±2.11 whereas, the lowest in halal slaughtered bird's meat were 10.09±1.10 and 870.48±2.11, respectively. However, Manganese (Mn) was only detected in halal slaughtered bird meat. Likewise, pH of dead chicken meat was lowest among treatments however, L* was highest in halal slaughtered bird meat. Additionally, the lowest lipid oxidation and haem iron values reported in halal slaughtered meat were 0.32±0.02 and 2.32±0.21, lower than in decapitated and dead bird meat. Our findings draw lines between slaughtering methods and mineral analysis could be used for the differentiation of halal slaughtered meats with dead chicken meat.

## PRACTICAL APPLICATION

Globally, meat adulteration is one of the major concerns in the world however, the situation is more grieving in developing countries like Pakistan. The adulteration of dead chicken meat with healthy broiler meat is reported and mainly used in low quality and cheap meat-based products by replacing quality meat with cheap ones. Questions regarding health issues is a special concern for consumers who have dietary restrictions due to religious beliefs and practices. Sophisticated techniques for the detection of adulterations involve the use of polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), amino acid analysis as well as using chromatography techniques but the major production bottlenecks associated with the use of these approaches need repeated results, intensive labor requirements and experience. In addition, these techniques are expensive, so we need time to make things easier and to take make fast decisions against this type of perishable foods, to seize the product or release it. The findings of this study could be beneficial for regulatory bodies to make quick and easy decisions against these types of violations as well as detect adulteration of halal meat with dead meat.

## INTRODUCTION

Food adulteration is a well-recognized serious problem worldwide and especially prevalent in the developing countries like Pakistan. It is an intentional activity from food producers and processors in which food quality is lowered either by the addition of low cost and inferior quality raw materials or by the removal of valuable ingredients from food products. Similarly, food adulterants like water, ice, dead animal meat as well as meat of other species of animals is added in the raw meat as meat based product that can decrease the product's quality as well as can harm consumers in terms of health and religious believes (Ortea *et al.*, 2012; Bansal *et al.*, 2017).

This type of adulteration is mainly due to the replacement of high-valued meat by cheaper ones. Moreover, economic aspects also raise questions regarding health issues as well as the matter of special concerns for consumers who have dietary restrictions due to ethnical options or religious practices (Ha *et al.*, 2017). Furthermore, certain groups of people do not eat specific foods because of their religious ethics and preferences among them. The Muslims are estimated to represent 1.8 billion of the total population of the world and are only allowed to consume halal meat. Additionally, the Muslims are expected to account for 31.1% of the world's population by 2060 (Amaral *et al.*, 2017).

Adulteration in food has been a concern since the beginning of civilization as it not only decreases food products quality but also results in a number of ill effects on health. The quality and authenticity testing and detection of adulterants in food products is required for product safety, value assessment, to assure consumer protection against fraud and to maintain the integrity and the quality of supply chain in food systems (Chuah *et al.*, 2016; Sohail, 2019). Food authenticity is an important criterion of food safety and quality for consumers and foods with Halal and Kosher certification are readily accepted by Muslim and Jewish consumers to whom the consumption of pork and its derivatives in any product is prohibited (Chaudry & Regenstein, 2003; Ali *et al.* 2011; Regenstein).

Global meat consumption continues to rise and is projected to increase by around 1.6 per cent a year from 2013 to 2022 (Farouk *et al.*, 2014; OECD/FAO, 2018). The poultry meat sector in Pakistan is well-organized compared to other meat sectors and it contributes 1.4% in gross domestic production (GDP). However, it's shear in agriculture, livestock and innovative meat products account for 6.9 to 11.7%. Poultry meat production was 767, 000 tons in 2010, after five years a significant increase recorded that the production reached 1074, 000 tons and is still mounting, which indicates the potential and the consumption of chicken meat (GOP 2018). The Halal food market currently accounts for 12% of the global trade in agro-food products. It is assumed that, halal foods are wholesome, as specified by religious texts, as well as hygienic and safe to eat. Its' certification protects against fraud of adulteration as well as meat and meat-based products are one of the major segment of halal food products produced locally as well as globally (Shahdan *et al.*, 2016; Knoll *et al.*, 2018).

Several slaughtering methods have been practiced worldwide; however dead chicken meat adulteration is a crucial issue in developing countries like Pakistan. Broiler chickens, which are a delicate living organism, die during transportation as well as when prone to stress conditions and its death can occur easily during the slightest shock of transportation (Ibrahim *et al.*, 2014). Some of the bird handlers as well as butchers do not discard these dead birds rather, they mix it with regular healthy chicken meat to be used for human consumption. This poses serious food safety and hygiene risks (Lambooij *et al.*, 2019; Martin *et al.*, 2019). The main risks to halal safety and hygiene associated with these actions, are health consequences as well as playing with Islamic belief of Muslim consumers (Zakaria, 2015; Sohaib & Jamil, 2017). In this context, there is dire need to develop parameters for the differentiation of slaughtered *vs*. dead chicken meat. Limited number of studies are available to compare composition, quality and safety traits of slaughtered chicken with dead chicken meat. Therefore, the present study conducted to determine compositional profiling, quality differences and safety attributes of the slaughtered chicken meat with dead chicken meat.

## MATERIAL AND METHODS

The present study was carried out at the Department of Food Science and Human Nutrition and Department of Meat Science, University of Veterinary and Animal Sciences, Lahore, Pakistan. In this study the birds were subjected to different slaughtering methods (halal vs non-halal) as well as dead chicken meat was examined for proximate composition, quality parameters as well as safety attributes. The study was conducted in triplicates and the birds were allocated in each group as per study plan mentioned in (Table 1).

**Table 1 –** Study plan.

| Treatments | Description |
|---|---|
| $T_1$ | Chicken birds subjected to halal slaughtering method |
| $T_2$ | Chicken birds subjected to decapitation slaughtering method |
| $T_3$ | Dead chicken bird meat not subjected to slaughtering method |

## Sample collection and preparation

Considering the study plan, broiler birds (48 broilers aged 6 weeks approximately, 2.4kg body weight) were purchased from a local market (Tollinton market as this is the largest market for distribution and supply of chicken in Lahore) ensuring that the birds were from the same lot to prevent interference of intrinsic and extrinsic factors. For slaughtering under aseptic conditions, the birds were taken immediately to the Department of Meat Science and subjected to halal and non-halal slaughtering methods (Ibrahim, Abdelgadir & Sulieman, 2014; Zaman *et al.*, 2017) followed by plucking and manual evisceration of the birds. Then meat samples were collected for analysis however, dead birds were not slaughtered and only de-feathered to make them ready for analysis. Afterwards, meat samples were packed to non-permeable polyethylene zip-lock bags followed by storage at 4°C for further proceedings (Fernandez, Forslid & Tornberg, 1994).

## Compositional analysis

### *Proximate composition*

The collected meat samples from dead birds and birds subjected to slaughtering (halal and non-halal) examined for proximate composition including moisture, dry matter, crude protein and crude fat, following the protocol of association of official analytical chemists described by (AOAC, 2010). For the purpose, moisture content determined using 10g sample in hot air oven at 100 °C for 8 hrs. The difference in weight among fresh and dehydrated samples characterized as water content were calculated using the following formula:

$$\% \text{ Moisture} = \frac{(\text{weight of wet sample-weight of dry sample})}{(\text{Weight of wet sample})} \times 100$$

For ash determination, samples were placed in muffle furnace at 650 °C for 4 hrs. After detonation the crucible was placed in the oven in middle high temperature for 30 min, and then cooled in the desiccator. Afterwards, the sample was weighed to constant weight and then ash was calculated using the following formula:

$$\%Ash = \frac{(\text{weight of crucible with cover + ash}) - \text{weight of crucile with cover}}{\text{original weight of sample}} \times 100$$

For crude protein**,** a sample of 1g was taken into a digestion flask followed by the addition of 5 g catalyst digestion mixture (1% ferrous sulphate: 5% copper sulphate: 94% potassium sulphate) and 30mL sulfuric acid. Afterwards, the sample digested to breakdown of organic material. Then the digested sample was subjected to neutralization and distillation; the digest was diluted with distilled water using a volumetric flask up to 250mL. Then 10mL of 4% boric acid was added into a 200mL receiving Erlenmeyer flask and 2 drops of indicator solution methyl red. The receiving flask was placed on the distillation system. 9mL of the digested sample was added to the 250mL volume flask and 10mL of 40% NaOH solution into the sample tube and steam generated by a flam lamp was started until the color of the receiving flask solution become yellow to golden. Titration was done using 0.1N $H_2SO_4$ in a burette and the volume of the acid used was utilized to calculate protein content using the following formula:

$$\%N = \left( \frac{(\text{Reading of used titor}(H_2SO_4) \in ml \times 0.1 \times 0.0014 \times 250}{10} \times 100 \right)$$

*Crude protein (CP) = % of N2 x 6.25*

For Crude fat, 2g accurately pre-dried sample was placed into an extraction thimble and n-hexane was used as the organic solvent for the extraction of crude fat in the boiling flask. The sample was placed in the thimble and set on a hot plate in high temperature in a Soxhlet extractor at a rate of 5-6 drops/Sec for about 4hrs. After the thimble dried in the hot air oven at 100° C for 30min, it was left to cool in the desiccator and was weigh. The fat content was calculated using the following formula:

$$\% \text{ Fat on dry weight basis} = \frac{\text{weight of sample + thimble after extraction}}{\text{weight of sample + thimble before extraction}} \times 100$$

## Mineral Analysis

The minerals (Iron, magnesium, copper, zinc and manganese) content were measured using Atomic Absorption Spectrophotometer in dead birds as well as poultry birds subjected to different slaughtering methods by following the guidelines of (Horwitz & Latimer, 2000). Purposely, 4g minced meat sample was mixed with 7mL of $HNO_3$ and 3mL $HCLO_4$ in an Erlenmeyer flask and the flask was placed on a hot plate until a crystal clear solution was formed indicating the completion of digestion. Afterwards, the digested sample was transferred into a volumetric flask made up to volume 10mL through deionized water. The mineral content was calculated as mg/kg f sample.

## Quality and safety evaluation of chicken meat samples

### *pH of meat samples*

The pH of dead and slaughtered chicken meat samples was determined using pH meter following guidelines of (Souza *et al.*, 2011). Accordingly, 10g of meat sample was homogenized in distilled water (90mL) and then transferred into a beaker and electrode along with a temperature probe. The reading appeared on pH meter was noted and recorded as pH value for samples.

### *Colorimetric analysis*

The CIE tristimulus (L* a*& b*) color values were determined using standardized calibrated Minolta® Chroma meter by following the protocol of (Rodriguez-Turienzo *et al.*, 2011). L* values determine lightness while a* measure redness (positive values indicate red color and negative indicates green color). However, b* measure yellowness of meat and meat products.

### *Cooking loss*

Cooking loss of chicken meat samples was documented by following the procedure of (Wong & Ashton, 2015). First of all, 150g of chicken meat samples prior to cooking was weighed followed by packing samples in polyethylene bags that were further subjected to heat in water bath (Memmert WNB45, Germany) maintained at 80° C. Afterwards, chicken meat samples were kept in water bath for cooking until the core temperature (measured using food grade thermometer) reached 72° C. After cooking, the samples were allowed to cool for 30 min at 4° C. After cooling, the samples weight was determined again to measure cooking loss which was calculated by using the following formula:

$$\text{Cooking loss \%} = \frac{\text{Weight before cooking} - \text{Weight after cooking}}{\text{Weight before cooking}} \times 100$$

## Texture analysis

The hardness of dead and slaughtered meat samples was determined using needle probe of texture analyzer (Imada® Co., Ltd. Japan) by following the method described by (Solomon, Eastridge, Paroczay & Bowker, 2008). Meat samples were placed under the needle of the analyzer and the amount of force was calculated. the The needle was applied perpendicularly to fiber direction and force applied to the meat sample showed hardness.

## Thiobarbituric acid reactive substances (TBARS) analysis

The oxidative stability of meat samples was determined using thiobarbituric acid reactive substances (TBARS) analysis following the guidelines of (Liu, Dai, Zhu & Li, 2010). Purposely, 5g meat samples were weighed in test tubes of 50mL and homogenization with butylated hydroxytoluene (BHT) 7.2% and 50µL deionized distilled water using a homogenizer for 15sec. A disposable test tube (13×100mm) having 1mL of homogenate and 2mL of thiobarbituric acid (TBA) trichloroacetic acid (TCA) 15 mM TBA/15% TCA. After that, boiling water bath was used for the incubation for 15min. Later, the samples were cooled in water bath for 10 min and then vortex again and centrifuged for 15min at 2000×g at 4°C. The absorbance of the resulting supernatant solution was determined at 531nm against a blank containing 1mL of deionized distilled water and 2mL of TBA/TCA solution. The TBARS value was calculated as milligrams of malondialdehyde (MDA) per kilogram of meat.

## Peroxide value determination

The peroxide value (PV) of chicken meat samples was determined using the method of (Rehman, Jingdong, Chandio & Hussain, 2017). Accordingly, 1g mined meat sample was mixed with 11mL of chloroform/ methanol ratio of 2:1 v/v followed by homogenization of the samples using (DLAB Homogenizer D-500) for 2 min at full speed. Afterwards, homogenation was conducted using whatman no.1 filter paper and then 7mL filtrate was followed by addition of 2 mL 0.5% NaCl. The mixture vortexed for 30 sec at full speed followed by centrifuge for 3 min at 3000g using (Hermle LaborTechnik GmbH - Z 300 K Universal Centrifuge, Germany). Afterwards, 3mL of lower phase was collected using micropipette and then added 2 mL cold chloroform/methanol 2:1 in test tube and then 25µL of 30% ammonium thiocyanate and the same quantity of 20 mM iron (II) chloride was added into it. The reaction mixture allowed standing at room temperature for 20 min followed by measuring absorbance at 500 nm by spectrophotometer.

## Heme iron content determination

Determination of heme iron content of chicken meat sample was determined as per method of minor amendment (4) (Addeen *et al.*, 2014). Minced, a 2g sample was mix with 9mL of acid acetone (HCl 2%, deionized water 8% and acetone 90% v/v/w). The blend was allowed to sit at room temperature and

was macerated by a glass rod. The mixture was then filtered with Whatman No. 42 and acid acetone was used as blank. The reading of the absorbance of the filtrate was at 640nm. The heme iron content mg/100g sample was calculated using the following formula:

Heme iron content (ppm) = Total pigment (ppm) x 0.0822

Where total pigment (ppm) = $A_{640}$ x 680.

### Non-haem iron content

The non-haem iron content of chicken meat sample was determined by following the guidelines of (Addeen, Benjakul, Wattanachant & Maqsood, 2014). Firstly, 1g of meat sample was placed into a screw cap test tube and then 50-µL sodium nitrite 0.39% w/v was added. Afterwards, freshly prepared 4mL mixture with ratio of 1:1 v/v of 40%vtrichloro acetic acid and 6N HCl were added. Then, capped tightly the tubes were set into a shaking incubator at 65ºC (Memmert, Germany) for 22 hrs and left to cool down at 25-30 ºC for 2 hrs. Freshly prepared 2mL of non-haem iron color reagent was mixed with 400 µL supernatant and vortexed at moderate speed. The mixture was then allowed to sit for 10 min at room temperature and the absorbance was recorded at 540nm using iron standard curve. The iron standard solution ranging (0-2 ppm) was used. Non-haem iron content was recorded as mg/100g sample. The color reagent was prepared with ratio of 1:20:20 w/v/v by following the protocol method of (Rehman *et al.*, 2017).

### Statistical analysis

The obtained data subjected to analysis of variance and mean of all parameters were compared using post hoc Duncan's test by following the guidelines of (Torrie & Steel, 1980) using Statistical Package for the Social Sciences (SPSS) 20 software (SPSS Inc., Chicago, IL, USA).

## RESULTS AND DISCUSSION

### Proximate composition of chicken meat

Meat quality always depends upon freshness and eating quality along with nutritional composition of meat that is considered a vital component as it provides information about vitamins and minerals along with macronutrients (Banović *et al.*, 2009). The statistical results regarding proximate composition analysis for slaughtered (Halal and decapitation/non-Halal) and dead chicken meat showed significant differences for ash and non-significant differences for moisture, crude protein as well as fat content (Table 2). Means regarding the elaborate moisture content was 71.77±2.03, 71.85±1.93 and 72.00±1.88 for birds subjected to halal slaughtering method ($T_{1)}$, birds subjected to decapitation slaughtering ($T_{2)}$ and dead bird meat ($T_3$), respectively, showed different values among the treatments. Likewise, crude protein content of chicken meat samples was 22.76±1.58, 22.10±1.68 and 21.78±1.60 for T1, T2 and $T_3$, respectively. Similarly, ash content of chicken meat samples was

**Table 2 –** Proximate composition of dead and broiler bird's meat subjected to different slaughtering methods.

| Treatments | Moisture | Dry Matter | Ash | Crude Protein | Crude Fat |
|---|---|---|---|---|---|
| $T_1$ | 71.77±2.03 | 27.8±1.93 | 1.11±0.16$^b$ | 22.76±1.58$^a$ | 3.54±0.36 |
| $T_2$ | 71.85±1.93 | 28.67±2.03 | 1.18±0.07$^a$ | 22.10±1.68$^a$ | 3.70±0.31 |
| $T_3$ | 72.00±1.88 | 28.00±1.88 | 1.26±0.31$^a$ | 21.78±1.60$^b$ | 3.77±0.26 |

Values are Means±SD. Means sharing similar superscript differ non significantly (p>0.05).

$T_1$: Chicken birds subjected to halal slaughtering method.

$T_2$: Chicken birds subjected to decapitation slaughtering method.

$T_3$: Dead chicken bird meat not subjected to slaughtering method.

1.11±0.16, 1.18±0.07 and 1.26±0.31 60 for $T_1$, $T_2$ and $T_3$, respectively.

The findings of this study are in line with one of the group of researchers who reported that moisture content varies from 61.21% to 59.51% for captive bolt stunning along with pithing and non-halal method of slaughtering while the animal is in death state (CSNHS) and captive bolt stunning along with no-pithing and halal method of slaughtering while animal is in live state (CSHS) group, respectively (Bostami *et al.*, 2018). A similar correlation between moisture content and muscular fat is observed. This is consistent with the previous study reported by (Bostami *et al.*, 2018; Fraser, 2008) where crude protein content is reported at 22.62% to 23.66% for the (CSNHS) and (CSHS) group, respectively.

### Minerals analysis chicken meat

The results showed statistically significant difference for minerals including iron (Fe), magnesium (Mg), copper (Cu), zinc (Zn) and manganese (Mn) in slaughtered and dead chicken

meat (Table 3). Means for Fe ($p$<0.05) in treatments $T_1$, $T_2$ and $T_3$ were as 10.09±1.10, 12.47±1.01 and 14.21±0.99mg/kg, respectively. Iron is the main component of blood, so the presence of Fe contents is another indication of residual blood presence in meat samples. Similarly, Mg ($p$<0.05) in treatments $T_1$, $T_2$ and $T_3$ were as 870.48±2.11, 930.79±2.11 and 959.62±2.11, respectively. Likewise, the highest copper documented in $T_2$ whereas, the same trend was documented in other treatments. The present study showed that Cu was 0.40±0.26, 0.46±0.30 and 0.40±0.26 for $T_1$, $T_2$ and $T_3$ respectively. The zinc level was also different and the maximum reported in $T_1$ (Halal slaughtered birds) as 55.98±2.17 whereas, minimum in $T_3$ (dead birds meat) as 24.80±1.75. Moreover, a unique trend was reported for Manganese in $T_1$ (Halal slaughtered chicken meat) 2.72±0.42 which was not reported among other treatments. Copper and iron can also act as pro-oxidants in broiler chicken meat. Alteration in metal ions, mostly Copper and iron, were recognized as major catalysts during the oxidation of lipids (Thanonkaew *et al.*, 2006). The outcomes suggested that bleeding throughout slaughtering process was varied. Iron content was found lowest in halal slaughtering treatment signifying a most efficient removal of blood. Throat cut was applied for halal treatment, in which major veins were cut to facilitate bleeding (Fraser, 2008; Ibrahim *et al.*, 2014). For dead treatment $T_3$, broilers died because of the stress. This meat contains large quantity of blood retained in the chicken carcasses which was the major source of pro-oxidants, iron contents particularly. Likewise, the second treatment $T_2$ (slaughtered by decapitation method) the rate of blood removal was reported but the rate was highest in the halal slaughtered birds' treatment and lowest in dead birds as indicated by the maximum Fe content in the muscles of the birds.

**Table 3 –** Minerals analysis of dead and chicken bird's meat subjected to halal and decapitation slaughtering methods.

| Treatments | Fe | Mg | Cu | Zn | Mn |
|---|---|---|---|---|---|
| $T_1$ | 10.09±1.10$^c$ | 870.48±2.31$^c$ | 0.38±0.06$^c$ | 55.98±2.17$^a$ | 2.83±0.42 |
| $T_2$ | 12.47±1.01$^b$ | 930.79±3.11$^b$ | 0.46±0.03$^b$ | 47.10±1.86$^b$ | ND* |
| $T_3$ | 14.21±0.99$^a$ | 959.62±3.81$^a$ | 0.67±0.05$^a$ | 24.80±1.7$^5$c | ND* |

Values are Means±SD. Means sharing similar superscript differ non significantly (p>0.05).

* Not detected=ND

T1: Chicken birds subjected to halal slaughtering method.

T2: Chicken birds subjected to decapitation slaughtering method.

T3: Dead chicken bird meat not subjected to slaughtering method.

## pH of broiler chicken meat

Food spoilage is caused mostly by microbes whose growth is mainly affected by factors like water, pH, oxygen, temperature and physical structure of food. Foods having neutral pH tend to spoil more rapidly than acidic foods, which are resistant to spoilage. Poultry meat has normal pH range between 5.9-6.2 while pH>6.4 describe dark, firm and dry meat (DFD) and pH<5.7 produces PSE meat after 15 minutes of slaughtering (Qiao *et al.*, 2001). Low pH (PSE) associated with higher drip loss (watery meat) and lighter meat color (vice versa for DFD). In addition, it is scientifically demonstrated that ritual slaughtering or the Islamic slaying method produced optimum pH meat, which is best for extended periods of storage as compared to other slaughtering meat (D'Agata *et al.*, 2009). The results (Table 4) indicated significant differences in meat pH due to different slaughtering methods. Means indicated highest pH in $T_1$ (Islamic slaughtering method) as 6.34±0.32 whereas, lowest in $T_3$ (dead

**Table 4 –** Physicochemical attributes including pH, Color, Cooking loss and texture of chicken meat samples subjected to different slaughtering methods.

| Treatments | pH | Color | | | Cooking loss | Texture |
|---|---|---|---|---|---|---|
| | | Lightness (L*) | Redness (a*) | Yellowness (b*) | | |
| $T_1$ | 6.34±0.32$^a$ | 52.78±2.35$^a$ | 14.46±2.11$^c$ | 11.63±1.57$^b$ | 21.23±1.95 | 7.55±0.45 |
| $T_2$ | 6.31±0.37$^a$ | 48.91±3.13$^b$ | 17.32±1.77$^{ab}$ | 12.44±2.46$^b$ | 22.17±4.69 | 9.17±0.02 |
| $T_3$ | 5.86±0.52$^b$ | 46.73±2.74$^c$ | 18.36±1.98$^a$ | 14.15±2.76$^a$ | 23.15±2.03 | 9.20±0.53 |

Values are Means±SD. Means sharing similar superscript differ non significantly (p>0.05).

$T_1$: Chicken birds subjected to halal slaughtering method.

$T_2$: Chicken birds subjected to decapitation slaughtering method.

$T_3$: Dead chicken bird meat not subjected to slaughtering method.

animal) as 5.86±0.52. In this study, pH of chicken meat sample was evaluated after slaughtering as well as de-feathering of unbled birds. The pH is an indicator of meat quality; usually low pH of meat concedes as poor quality of meat as leads to poor water holding capacity and more cooking loss and soft texture of raw meat (Fernandez *et al.*, 1994). There was a difference in the pH among treatments however; pH of $T_3$ was significantly lower compared to others. The decrease in pH was due to the increased production of lactic acid and Adenosine triphosphate (ATP) exhaustion in muscles caused by postmortem glycolysis. Similarly, Thomson *et al.*, (1986) reported struggling by birds during slaughtering affect postmortem glycolysis. They carried out a study to check the effects of electrical stunning prior to death and hot deboning on pH. They reported breast meat from birds stunned prior to death exhibited higher pH at 20 min postmortem than meat from non-stunned birds. Another probable reason of the high pH may be related to residual blood in the carcass (Hafiz *et al.*, 2015). Hence, the slaughtering process had potential effect on the pH of broiler meat.

## Color of chicken meat

Meat quality includes measurement of attributes that determine suitability of meat to be eaten as fresh or stored for a reasonable period without deterioration (Elmasry *et al.*, 2012a). The first important parameter to estimate meat quality is the color of meat, which is the base of meat selection and acceptability by the consumer. That is why comprehensive knowledge of variations in color of meat is needed to avoid further negative impact during processing of the meat product. Color of meat samples mostly measured by lightness (L*), redness (a*) and yellowness (b*) is one of the criteria that influence the consumer's choice as well as indicator of the meat quality. In addition, different treatments have significant effect on meat color, which expressed quality due to high blood retention within the meat tissues. Results reported significant change in L* value with mean 52.78±2.35, 48.91±3.13 and 46.73±2.74 for $T_1$, $T_2$ and $T_3$, correspondingly (Table 4). The highest mean was documented in $T_1$ compared to other treatments. Redness (a*) of meat is another indication of presence of residual blood content. Results reported highest value in T3 as 18.36±1.98 that indicate the presence of the highest levels of blood whereas, the lowest in T1 was 14.46±2.11. Likewise, yellowness is also one of the attributes in chicken meat color. The chicken meat of halal slaughter treatment reported lowest mean for this trait as 11.63±1.57 whereas, T3 documented the highest mean as 14.15±2.76. Once compared the mean value of halal slaughter with other treatments that shows lowest redness (a*) and higher a* observed in unbled samples might be due to blood retained in meat samples. One of the researcher's groups reported a* value interrelated with total pigment, myoglobin and Fe content in meat. Halal slaughtered meat samples reported lowest a* and b*value. Yellowness is also considered as the quality factor of chicken meat. The chicken for halal slaughter treatment had the lowest level compared with other treatments that is the symbol of lowest level of residual blood contents. The dead meat animals reported highest yellowness due to highest residual blood contents and post-mortem changes that is also reported by (Schreurs, 2000; Ali *et al.*, 2011).

## Texture and cooking loss of chicken meat

The texture of chicken meat shows non-significant ($p>0.05$) differences between treatments (Table 4). The means of shear force values were 7.55±0.45, 9.17±0.02 and 9.20±0.53 for $T_1$, $T_2$ and $T_3$, respectively. At some stages of post-mortem proteolysis, intracellular meditation of calcium raises and stimulates calcium activated enzymes, which is assumed for attacking contractile proteins. Afterwards releasing the arrangement of muscle non-enzymically which leads to tenderization (Fraser, 2008). Similarly, cooking loss of chicken meat also showed non-significant ($p>0.05$) differences among treatments (Table 4). Means for cooking loss were 21.23±1.95, 22.17±4.69 and 23.15±2.03 for $T_1$, $T_2$ and $T_3$, respectively. However, the lowest cooking loss % was reported in T1, that was slaughtered by halal slaughtering method also indicates good quality. The low water holding capacity of chicken meat is a condition showing protein denaturation (Cumby *et al.*, 2008; Zhuang *et al.*, 2013). Halal slaughtered meat samples exhibited lower cooking loss, related to high WHC as compared to non-Halal slaughtering method. In previous studies, Addeen *et al.* (2014) observed WHC by cooking loss in chicken meat samples slaughtered with different methods. Meat samples obtained after Halal slaughtering showed a lower drip loss value *i.e.* water binding properties of muscle, which increase with storage time (Addeen *et al.*, 2014; D'Agata *et al.*, 2009). In contrary, Hafiz *et al.* (2015) suggested no significant difference in cooking and thaw losses for Chinese slaughtered meat and Islamic slaughtered meat. However, Islamic slaughtered meat indicated less water lost during thawing and cooking process. In addition, when comparison was made for dead

bird's meat with other treatments, $T_2$ and $T_1$ showed highest cooking loss. Through heat, water inside the meat tissues situated in the thin canals between the filaments was released as meat contracts (Bertola *et al.*, 1994).

## Lipid oxidation of broiler chicken meat

Lipid oxidation is the major cause responsible for deterioration in meat and meat products. This process is mainly initiated in ferric heme pigments implicated as pro-oxidants in living tissues. The results regarding thiobarbituric acid reactive substances (TBARS) and peroxide value (PV) of broiler meat subjected to slaughtering as well as dead bird's meat showed significant differences. Means of TBARS for treatments showed the highest value for $T_3$ (dead chicken meat) was 1.27±0.02 whereas, lowest in T1 (Halal slaughtered chicken meat) as 0.32±0.02. Similarly, the peroxide value showed similar trend as reported by TBARS analysis. Highest PV documented in $T_3$ (dead chicken meat) was 1.27±0.02 whereas, lowest reported in $T_1$ (Halal slaughtered chicken meat) as 0.32±0.02. Moreover, PV in $T_2$ (chicken meat subjected to decapitation slaughtering) was 0.59±0.03. The higher TBARS and PV in dead meat denote spoilage process has been initiated in chicken meat that has contributed towards higher TBARS value.

Lipid oxidation is a well-known indicator of meat quality, usually high level of meat concedes as poor quality of meat. The results were comparable in all samples ($p$<0.05) pointed toward formation of pro-oxidants resulting in the increase of lipid oxidation in dead birds and lowest in Halal slaughtered chicken meat samples. These findings are supported by pervious researches which reported that higher levels of retained blood contents in the muscles lead to more lipid oxidations (Alvarado *et al.*, 2007). Similarly, other researchers group also stated that blood retained in meat samples along with other constituents like white cells, produce oxides and radicals that ultimately promote lipid oxidation. In addition, Non-heme iron is also known as the effective catalyst of lipid oxidation in muscles. (Addeen *et al.*, 2014) stated that copper and iron could also act as pro-oxidants in broiler meat. Alteration in metal ions, mostly Copper and iron, were recognized as major catalysts during oxidation of lipids, which are found to lowest levels in halal slaughtered meat samples.

## Haem and non-haem iron content

Iron has an advantageous effect on certain organoleptic characteristics of meat because it is a key component of myoglobin responsible for color of meat (Lucke *et al.*, 2017). The results regarding haem and non-haem content of broiler chicken meat subjected to halal and non-halal slaughtering as well as dead birds showed significant ($p$>0.05) differences. Means for haem reported maximum value in $T_3$ (dead chicken meat) as 3.11±0.18 whereas, minimum in $T_1$ (Halal slaughtered chicken meat) as 2.32±0.21. However, heam iron in $T_2$ (chicken meat subjected to decapitation method) was 2.41±0.16. Similarly, non-haem iron was 0.04±0.0002, 0.07±0.0001, 0.11±0.0003 and in different treatments $T_1$, $T_2$ and $T_3$, respectively.

Haem and non-haem iron contents shown significant results between treatments ($p$<0.05) and correlated to meat quality factors *i.e.* residual blood level measured by their concentration. The findings of instant study coincide with the research of (Addeen *et al.*, 2014) who also reported higher values in non-halal slaughtered meat samples whereas, lower in halal slaughtered meat due to efficient blood removal. The blood includes large amount of hemoglobin consisting of 4 polypeptides with every chain containing haem group and each haem contains 1 iron atom corresponding within porphyrin ring (Richards *et al.*, 2007). The unconfined Fe can motivate lipid oxidation of muscle. Similarly, (Richards *et al.*, 2007) also stated that higher hemoglobin reported in broiler chicken meat is not subjected to halal slaughtering than their counterparts ($p$<0.05). When samples of dead and slaughtered meat are compared, noticeable increase in non-haem iron content due to storage as reported by (Addeen *et al.*, 2014) are seen. Non-haem iron is

**Table 5 –** Thiobarbituric acid reactive substances, Peroxide value, Haem and Non-Haem iron of chicken meat samples.

| Treatments | TBARS Value | Peroxide Value | Haem Iron | Non Haem Iron |
|---|---|---|---|---|
| $T_1$ | 0.32±0.02$^b$ | 0.36±0.01$^b$ | 2.32±0.21$^b$ | 0.04±0.0002$^c$ |
| $T_2$ | 0.59±0.03$^b$ | 0.36±0.03$^b$ | 2.41±0.16$^b$ | 0.07±0.0001$^b$ |
| $T_3$ | 1.27±0.02$^a$ | 0.68±0.04$^a$ | 3.11±0.18$^a$ | 0.11±0.0003$^a$ |

Values are Means±SD. Means sharing similar superscript differ non significantly ($p$>0.05).

$T_1$: Chicken birds subjected to halal slaughtering method.

$T_2$: Chicken birds subjected to decapitation slaughtering method.

$T_3$: Dead chicken bird meat not subjected to slaughtering method.

also known as the effective catalyst of lipid oxidation in muscles. Likewise, also stated qualitative factors related to deterioration of sub-cellular organelles *i.e.* mitochondria and release of cytochrome c leads to the enhancement of soluble haem in meat samples leading towards spoilage (Decker & Hultin, 1990).

## CONCLUSION

Poultry birds are very delicate and prone to stress conditions, thus death occurs easily during slight shock of transportation. The meat of this dead bird can become the part of food supply chain by producers, handlers as well as butchers due to malpractices. Also, the major risks associated with this dead bird's meat can affect consumer's health. The present study's findings indicated significant changes in protein and ash content of slaughtered and dead bird's meat. Likewise, chicken meat from halal slaughtering method depicted lower heme and non-heme iron as well as lower lipid oxidation than the decapitation slaughtering method and of dead meat. Also, mineral analysis provided useful results for the differentiation of dead and slaughtered meat that could be used in the future by the regulatory agencies to maintain safety and quality of food supply chain in Pakistan.

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## CONFLICTS OF INTEREST

The authors report no conflict of interest.

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