



Rigor mortis and feather plucking resistance as external markers for post-mortem interval estimation in chicken[#]

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Abstract

Accurate estimation of the post-mortem interval (PMI) in avian species remains challenging due to rapid post-mortem changes and the limited availability of validated external indicators. The present study evaluated the progression of rigor mortis and feather plucking resistance as practical markers for PMI estimation in chickens. A total of thirty-six adult spent chickens were humanely euthanised and examined at defined post-mortem intervals. Rigor mortis was assessed at 0.5, 1, 2.5, 6, 12, 24 and 36 hours PMI. Six birds were removed randomly from the observations at 0, 6, 12, 24, 48 and 72 hours PMI. The feather plucking resistance of these birds were evaluated using a ranked feather plucking resistance scale measured at each interval. Rigor mortis showed a consistent and time-dependent progression, beginning in the legs and extending to the beak region and wings, with complete rigidity observed within 12 hours. Partial resolution was evident at 24 hours, and complete disappearance by 36 hours. A very strong positive correlation was observed between PMI and rigor mortis progression (Spearman's $\rho = 0.948$, $p < 0.001$). The resistance needed for feather plucking declined noticeably around 24 hours and substantial reduction between 48 and 72 hours PMI. Spearman's rank correlation analysis revealed a strong and statistically significant inverse relationship between post-mortem interval and feather plucking resistance ($r^2 = -0.90$, $p < 0.001$). Rigor mortis was found to be a useful indicator for estimating the post-mortem interval up to 12 hours while partial resolution at 24 hours and complete disappearance by 36 hours facilitated differentiation between early and intermediate post-mortem stages. Feather plucking resistance served as a useful complementary indicator, extending PMI estimation up to 72 hours. However, these parameters should be interpreted with caution, considering the influence of environmental conditions, tissue degradation and post-mortem biochemical changes.

Keywords: Post-mortem interval, Rigor mortis, Feather plucking resistance, Chicken

Post-mortem interval (PMI), defined as the time elapsed since death, is a critical parameter in forensic and veterinary investigations (Wenzlow et al., 2023). In avian species, its estimation is challenging due to rapid post-mortem

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biochemical changes and limited validated indicators (Valverde et al., 2020). Conventional methods based on observable changes, such as body cooling and rigor mortis, remain widely used due to their simplicity. Rigor mortis, resulting from ATP depletion and actin–myosin cross-linking, leads to muscle stiffening and serves as a reliable indicator of early PMI (Gupta et al., 2025). In contrast, external morphological changes such as feather attachment strength have received limited attention despite their potential forensic value. Feather anchorage reflects the integrity of the skin–follicle complex and may decline with progressive tissue degradation (Lucas and Stettenheim, 1972). Studies on PMI estimation in chickens using these parameters under Indian conditions are limited. Therefore, the present study aimed to evaluate rigor mortis and feather plucking resistance for PMI estimation in chickens.

Materials and methods

The present study was conducted at the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Pookode, Wayanad. A total of thirty six adult spent chickens, each weighing approximately 1.5 kg, were utilised for the study. The birds were humanely euthanised by cervical dislocation and maintained under ambient room conditions in a well-ventilated environment. Cervical dislocation is widely used as a humane method for euthanasia in birds (Bandara et al., 2023). The study was conducted following approval from the Institutional Animal Ethics Committee (IAEC), College of Veterinary and Animal Sciences, Pookode (Approval No.: IAEC/COVAS/PKD/24/18/2025; dated 12.03.2025).

The study followed a longitudinal observational design involving repeated post-mortem assessment of the same birds over time for rigor mortis. Rigor mortis in the beak region, wings, and legs was evaluated at 0.5, 1, 2.5, 6, 12, 24 and 36 hours post-mortem interval (PMI). A total of 36 birds were initially included in the study, and six birds were sequentially removed at 0, 6, 12, 24, 48 and 72 hours post-mortem for additional experimental purposes. As rigor mortis was not assessed at 0 hours post-mortem, the remaining 30 birds constituted the initial cohort for rigor mortis evaluation. Birds selected for subsequent experimental procedures were assessed for rigor mortis at the respective post-mortem interval prior to removal from further observation, resulting in a progressive reduction in sample size during later observation periods. The presence or absence of rigor mortis in each anatomical region was determined by applying gentle manual pressure and evaluating the degree of stiffness and flexibility, which was categorised as either rigid or absent. Resolution of rigor mortis was identified by the return of flexibility upon manual examination. Statistical analysis was performed using all available rigor mortis observations recorded at each post-mortem interval from the birds available at the respective time points.

Feather plucking resistance was assessed at 0, 6, 12, 24, 48, and 72 hours post-mortem using six birds at each respective time point, following a cross-sectional study. Feathers were manually removed from the abdominal region, which was selected due to its uniform feather distribution and ease of access, thereby ensuring reproducible sampling. To maintain consistency, all feather plucking procedures were performed by a single trained observer following a standardised protocol established for the study, which involved maintaining a uniform angle, direction, and steady pulling force along the axis of feather growth. Feathers were manually extracted from the abdominal region by grasping them close to the skin and applying a steady, uniform force in the direction of feather growth. Feather plucking resistance was graded using a ranked scale from 4 to 1, where a score of 4 represented maximum resistance immediately after death and a score of 1 represented minimal resistance. Statistical analysis was performed using observations obtained from six birds at each respective time point.

The relationships between PMI and rigor mortis progression, as well as feather plucking resistance were analysed using Spearman's rank-order correlation. Correlation analysis was performed using the Statistical Package for Social Sciences (SPSS) version 20.0. A p-value of less than 0.05 was considered statistically significant.

Results and discussion

Assessment of rigor mortis was performed across different body regions, namely the beak region, legs and wings. The progression of stiffness among these regions showed temporal variation.

At 0.5 hours post-mortem, rigor mortis was predominantly observed in the legs alone (60%, $n=18$). Concurrent involvement of both the legs and beak region was noted in a smaller number of birds (20%, $n=6$), while only one bird (3.33%) exhibited stiffness limited to the beak region. The remaining birds (16.67%, $n=5$) showed no evidence of rigor mortis at this stage.

At 1 hour post-mortem, rigor mortis showed a clear progression compared to earlier time points, with involvement of a larger number of anatomical regions and a wider spread across the body. The most common pattern observed was the simultaneous involvement of the leg and beak region (50%, $n = 15$), indicating that rigor mortis had progressed beyond the initial localised stage seen at earlier time point. The proportion of birds showing rigour restricted only to the leg decreased to 20 per cent ($n = 6$), suggesting that rigidity, which initially develops in the leg muscles, progressively extends to additional anatomical regions over time. Notably, all five birds that exhibited no rigor at 0.5 hours developed rigor mortis confined to the legs by 1 hour. In addition, a smaller proportion of birds showed stiffness of multiple anatomical regions, including

the leg and wing without stiffness of the beak region (16.67%, $n = 5$), as well as simultaneous stiffness of the leg, beak region, and wing (13.33%, $n = 4$). No birds exhibited stiffness limited exclusively to the beak region, and no cases of complete absence of rigor were observed at this stage.

At 2.5 hours post-mortem, rigor mortis became mainly generalised, with involvement of the leg, beak region, and wing observed in most birds (83.33%, $n=25$). Fewer birds showed involvement of the leg and beak region (10%, $n=3$) and 2 birds showed rigour involving leg and wing only, without involvement of beak region (6.67%, $n=2$).

At 6 hours post-mortem, 90 per cent ($n=27$) birds exhibited complete rigor mortis involving all anatomical regions. The two birds that showed rigor involving the leg and wing at 2 hours progressed to complete rigor involving the leg, beak region, and wing by 6 hours. In contrast, the three birds that exhibited rigor limited to the leg and beak region at 2.5 hours remained in the same condition at 6 hours, without additional involvement of the wing.

At 12 hours post-mortem, all examined birds ($n = 24$) exhibited generalised rigor mortis affecting the legs, beak region, and wings. At 24 hours post-mortem, the beak region and legs of all birds ($n = 18$) were movable with moderate resistance, indicating partial resolution of rigor mortis; however, the wings remained rigid and stiff. By 36 hours post-mortem, rigor mortis had completely resolved in all anatomical regions. Details of region-wise rigor mortis at different PMI are given in Table 1.

The progression of rigor mortis from initial involvement of the leg to subsequent involvement of the beak region and wing proved to be a useful indicator for estimating PMI during the early post-mortem period. The onset of rigor mortis in the leg was observed as early as 0.5 hours and as late as one hour post-mortem. Similarly, beak region stiffening was observed as early as 0.5 hours and as late as one hour. In contrast, stiffening in the wing

was observed as early as one hour and as late as 12 hours post-mortem.

The progression of rigor mortis over the first 12 hours post-mortem represents a well-defined phase for determining the post-mortem interval. The orderly involvement of anatomical regions, initially affecting the leg and subsequently extending to the beak region and wings, demonstrates a consistent pattern useful for early PMI assessment. The initiation of rigor resolution at approximately 24 hours, followed by its complete disappearance by 36 hours, aided in distinguishing between early and intermediate stages of the post-mortem interval.

Spearman's rank-order correlation demonstrated a very strong positive association between post-mortem interval and rigor mortis rank among the birds available at the respective observation periods (Spearman's $\rho = 0.948$, $p < 0.001$; $n = 174$ observations), indicating a highly ordered and time-dependent progression of muscular rigidity.

In an earlier study on ducks, rigor mortis was noted to develop initially in the neck and leg muscles within one hour after death, whereas stiffness in the pectoral muscles was reported to occur later, at around 1.5 hours post-mortem (Morrow and Glover, 1970). A comparable trend was observed in the present investigation, where the earliest signs of rigor mortis were detected in the legs at approximately 0.5 hours following death. At 1 hour post-mortem, stiffening progressed with combined involvement of the leg and beak region in most birds. In raptors, stiffening was observed within 1 hour in the neck and legs, while it was less appreciable in the beak region, with beak region involvement becoming evident around 1.5 hours, followed by complete rigor by 2.5 hours (Valverde et al., 2020). A similar pattern was observed in the present study, where rigor mortis in the legs was evident in all birds within 1 hour. Beak region stiffening was observed in 20 birds within the same period, whereas involvement of the wings occurred later, between 1 and 6 hours post-mortem.

Table 1. Rigor mortis status in chicken carcasses by anatomical region at various post-mortem intervals

PMI (hours)	Leg only	Beak region only	No rigor	Leg + Beak region	Leg+ Wing	Leg +Beak region +Wing	Residual Wing rigor	Total observed birds
0.5	18	1	5	6	0	0	0	30
1	6	0	0	15	5	4	0	30
2.5	0	0	0	3	2	25	0	30
6	0	0	0	3	0	27	0	30
12	0	0	0	0	0	24	0	24
24	0	0	0	0	0	0	18	18
36	0	0	0	0	0	0	0	12
Cumulative repeated observations								174

According to Oates et al. (1984), the onset of rigor mortis in the leg muscles of geese was reported to occur as early as 8 minutes and as late as 45 minutes post-mortem. In the present study, rigor mortis in the leg was observed within a slightly later range, appearing within 0.5 hours after death for 18 birds. This relatively rapid onset is comparable to findings in dogs, where rigor mortis was reported to begin within 2.5 minutes post-mortem (Abdou, 2001).

In the current study, the resolution of rigor mortis commenced at around 24 hours and was completed by 36 hours, indicating a shorter duration compared to humans. In humans, rigor mortis typically begins within 3 to 4 hours after death, reaches peak stiffness between 6 and 12 hours, persists for 18 to 36 hours, and is followed by secondary flaccidity (Madea, 2016). In contrast, studies in raptors have reported a more prolonged persistence, with reduction becoming more evident by 48 hours and complete disappearance occurring by 72 hours (Valverde et al., 2020).

Based on the present investigation, rigor mortis can be effectively utilised for estimating the PMI during the early post-mortem period, particularly up to 12 hours. The orderly progression of rigor mortis across different anatomical regions provides a reliable and time-dependent indicator for PMI assessment. However, the onset, progression, and duration of rigor mortis are influenced by multiple factors, including glycogen reserves, species differences, body size, environmental conditions, and the physiological state at the time of death. These variables may significantly alter both the timing of onset and the extent of post-mortem muscular stiffening, and should therefore be carefully considered when interpreting rigor mortis for PMI estimation.

Feather plucking resistance was graded on a scale from 4 (observed just after death) to 1, representing decreasing resistance to plucking. To ensure consistency, all feather plucking was performed by a single trained observer using a lab standardised technique that involved maintaining a uniform angle, direction, and steady pulling resistance along the axis of feather growth. Feathers were extracted individually from each bird at each time point by grasping close to the skin and applying a steady, uniform resistance along the direction of growth. Feather attachment strength remained relatively stable during the early post-mortem period (0–12 hours), followed by a noticeable reduction at approximately 24 hours. At 24 hours post-mortem, two birds maintained strong resistance to plucking (score 4), in contrast to the remaining four birds in the same group, which exhibited lower resistance.

Birds of similar age and body weight were selected to minimise biological variation. A marked decline in feather attachment strength was observed between 48 and 72 hours. Spearman's rank correlation analysis demonstrated a strong and statistically significant inverse

relationship between post-mortem interval and feather plucking resistance ($r(36) = -0.90, p < 0.001$).

Table 2. PMI and Feather plucking resistance

PMI (hours)	No of birds observed	Feather Plucking Resistance
0	6	4
6	6	4
12	6	4
24	4	3
24	2	4
48	6	2
72	6	1

The results demonstrated that, with increasing post-mortem interval (PMI), the resistance required for feather plucking decreased significantly. A pronounced reduction in feather retention was evident by 48 hours PMI, likely due to the degradation of collagen-rich dermal tissues that anchor the feather shaft within the integument (Lucas and Stettenheim, 1972). The slight variation observed at 24 hours, particularly in two birds, may be attributed to individual differences in enzymatic activity, resulting in delayed breakdown of follicular connective tissue. The persistence of mild resistance to feather plucking even at 72 hours PMI may serve as a useful indicator for PMI estimation in chickens. However, factors such as environmental conditions and tissue variability should be carefully considered when interpreting these findings. The feather plucking scoring system used was observational and requires further validation through objective quantitative methods.

Conclusion

Based on the present investigation, rigor mortis showed a consistent and time-dependent progression across anatomical regions and may serve as a useful indicator for post-mortem interval (PMI) estimation during the early post-mortem period, particularly up to 12 hours. The sequential involvement of the leg, followed by the beak region and wings, provided a practical framework for early PMI assessment, while the onset of resolution at approximately 24 hours and its complete disappearance by 36 hours assisted in distinguishing intermediate post-mortem stages.

Feather plucking resistance demonstrated a progressive decline with increasing PMI and may serve as a complementary indicator for PMI estimation up to 72 hours post-mortem. However, this parameter should preferably be interpreted alongside other post-mortem findings, as it may be influenced by physiological condition, environmental factors, tissue degradation, and technique-related variations. The feather plucking scoring system used was observational and requires further validation through objective quantitative methods.

Overall, the combined assessment of rigor mortis and feather plucking resistance may improve the practical estimation of PMI in chickens under the present experimental conditions. Nevertheless, further standardization and validation of feather plucking assessment using objective quantitative methods are recommended to improve repeatability and reduce observer-related variation.

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Conflict of interest

The authors declare that there is no conflict of interest.

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