











Age and body weight as determinants of semen quality and sperm motility in Ross 308 broiler breeder males

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ABSTRACT - Rooster fertility has a considerable impact on reproductive efficiency in broiler breeder management, with semen quality being an important factor in fertilization success. This study evaluated the effects of age and body weight on semen quality measures in Ross 308 broiler breeder males. Twenty-four roosters were allocated into two age groups (25-29 weeks and 30-39 weeks) and three body weight categories (underweight: 3.0-3.5 kg; normal: 4.0-4.5 kg; overweight: 5.0-5.5 kg). The volume, concentration, motility, and viability of sperm samples were evaluated. Sperm motility was affected by age and body weight. Older roosters showed higher motility (69.33%) than younger ones (41.26%). Roosters with normal body weight had better sperm motility (84.0%) than roosters that were underweight (44.17%) or overweight (46.33%). Semen volume increased with age only in overweight roosters, whereas other parameters were not significantly affected. These findings underline need to maintain an optimal body weight to optimize fertility, while also acknowledging the beneficial effects of advancing age on sperm motility. Age-specific weight management strategies may improve reproductive performance in broiler breeder males.

Keywords: male performance, poultry, reproductive traits, sperm quality



1. Introduction

Productivity in broiler breeder operations is largely determined by reproductive efficiency since male fertility directly affects fertilization success, hatchability rates, and overall flock performance (Ncho et al., 2025). Roosters' fertility depends mainly on semen quality, which is defined by sperm concentration, motility, viability, and volume. Maximizing fertilization rates depends on maintaining good semen quality, especially in industrial chicken farming systems where natural mating is still the major technique of reproduction (Wang et al., 2024). Although environmental variables, diet, and genetic selection affect semen quality, two key factors that can substantially influence reproductive function are age and body weight (Tadondjou et al., 2013; Gonçalves et al., 2015; Ayeneshet et al., 2024).

Age-related changes in semen traits have been recorded in broiler breeder males; older roosters often show better sperm motility because of the development of their reproductive system and hormonal stability (Barbarestani et al., 2025). Advancing age, however, can also lead to oxidative

stress and cellular damage, which may reduce sperm viability and overall semen quality (Haryuni et al., 2022). Furthermore, body weight is a critical factor for reproductive performance since both underweight and overweight states can impair sperm production and function. Underweight rooster may have nutritional deficiencies that affect spermatogenesis, therefore lowering sperm count and compromising semen quality (Shaheen et al., 2023). On the other hand, overweight roosters are sometimes associated with increased fat deposition, hormonal imbalances, and decreased testicular efficiency, which can adversely influence sperm motility and fertility outcomes (Olubowale, 2013).

The combined effects of age and body weight on semen quality in male broiler breeders are still not well understood, despite these findings. Previous research has focused on the individual effects of age or weight, although understanding their interaction is vital for creating effective management strategies to improve reproductive success. Inadequate weight control in breeding males can lead to suboptimal semen production, thereby reducing the fertilization success and overall flock fertility (Oke et al., 2024). Therefore, examining the interaction between age and body weight in broiler breeder males is required to identify the ideal weight range and age for optimizing semen quality and fertility potential. Although prior research has examined the separate effects of age and body weight, there is limited information regarding their interaction in tropical environments, particularly for Ross 308 breeder males. This study offers new insights into the interactions between age and weight on semen characteristics under equatorial management systems.

Notwithstanding the available evidence regarding the separate impacts of age and body weight on semen characteristics, there is still limited information regarding their synergistic effects in broiler breeder males, especially in commercial strains such as Ross 308. Addressing this issue is essential for enhancing male reproductive efficiency through optimized management strategies. This study aimed to evaluate the interactive effects of age and body weight on semen quality parameters in Ross 308 broiler breeder males. The evaluated parameters included sperm viability, sperm motility, sperm concentration, and semen volume. The findings are expected to support the identification of optimal age-weight combinations that maximize fertility potential and semen quality. It is hypothesized that semen characteristics are significantly influenced by both age and body weight, as well as by their interaction. Specifically, roosters with normal body weight at older ages will demonstrate superior semen quality and sperm motility compared with other age-weight combinations.

2. Material and methods

The study was conducted at the Faculty of Sustainable Agriculture, Sandakan, Sabah, Malaysia (5°56' north latitude 118°00' east longitude, with altitude of 25 m with a predominant tropical rainforest climate).

After approval by the Universiti Malaysia Sabah Animal Ethics Committee (JEHUMS), experimental procedures were implemented in accordance with the Researchers' Guidelines on the Code of Practice for the Care and Use of Animals for Scientific Purposes (Approval No. AEC 0012/2024).

Twenty-four Ross 308 broiler breeder males were randomly assigned to six experimental groups, each consisting of four roosters, and were confined in wire-floored metal cages (65 × 55 × 70 cm), individually placed in a naturally ventilated poultry house. The facility was constructed to allow for adequate airflow and exposure to ambient environmental conditions, including natural daylight. Birds were managed under a natural photoperiod without artificial lighting, with day length ranging approximately from 12 to 13 h throughout the study period. The experimental treatments comprised two age groups (25-29 weeks and 30-39 weeks) and three weight classifications: normal (4.0-4.5 kg), underweight (3.0-3.5 kg), and overweight (5.0-5.5 kg). Body-weight classifications were determined based on the Ross 308 Performance Objectives manual Ross 308 FF Parent Stock: Performance Objectives (Aviagen, 2011).

The roosters received commercial male broiler breeder pellets containing 13% moisture, 13% crude protein, 3.5% crude fat, 7% crude fiber, 4% calcium, and 0.7% phosphorus. Feed and water were

provided *ad libitum*. Each rooster was provided with approximately 110-120 g of feed daily, adjusted according to its respective body weight group. Feed was provided once daily at 06:00 h.

Prior to data collection, all birds underwent a two-week acclimatization period, during which they were conditioned for semen collection using the abdominal massage method (Burrows and Quinn, 1937). Semen was obtained from each rooster, and individual sperm evaluation were performed. Health status and general behavior was monitored twice daily throughout the study. Cage trays were cleaned weekly, and accumulated waste was removed daily to maintain hygiene.

Volume was measured immediately after semen collection using a 1 mL syringe. The evaluation of color was conducted using the color score, 1 - Watery, 2 - Cloudy and 3 - Creamy (Mohamad Asrol and Abdul Rashid, 2017).

The sperm concentration of an output was assessed using a hemocytometer chamber following a dilution with distilled water at a 1:400 ratio, expressed as $\times 10^9 \text{ mL}^{-1}$.

A drop of undiluted semen was applied to a microscope slide without a coverslip, and mass motility was evaluated by examining it under a compound microscope at 100 \times magnification. A scale of 1 to 5, based on Pimprasert et al. (2023) was used to assess motility.

According to recognized protocols, semen was diluted using a modified Ringer's solution at a 1:100 ratio (semen:extender) for progressive motility (Mohamad Asrol and Abdul Rashid, 2017). A drop of diluted semen was examined under a microscope at 400 \times magnification after being placed on a slide and covered with a glass coverslip. The proportion of motile spermatozoa exhibiting moderate to rapid progressive movement was utilized to assess motility. Each sample was analyzed in at least three microscopic fields.

The eosin-nigrosin stain method was used to evaluate the sperm viability (Mohamad Asrol and Abdul Rashid, 2017). An equal volume of eosin-nigrosin stain was well mixed with a 10 μL sample of semen. The mixture was then spread uniformly on a microscope slide and allowed to air-dry. A phase-contrast microscope at 400 \times magnification was used to view the slide. A total of 200 spermatozoa per sample were evaluated. Unstained sperm were classified as viable, whereas stained sperm were regarded as nonviable.

Data were analyzed using SAS[®] OnDemand for Academics version 9.4 statistical software (SAS Institute Inc., 2024). Initially, the data were assessed for normality and homogeneity of variance, followed by analysis using PROC GLM under a completely randomized design (CRD). The statistical model included the main effects of age and body weight, as well as their interaction (age \times body weight). When a significant interaction was detected, simple-effect comparisons were performed using Tukey's post-hoc test to examine differences within age or body weight classes. Differences were deemed significant when $P < 0.05$. The impact of age and weight was assessed using the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha \times \beta)_{ij} + e_{ijk} \quad (1)$$

in which Y_{ijk} is the quantitative response variable, μ is the overall mean, α_i is the fixed effect of age, β_j is the fixed effect of weight, $(\alpha \times \beta)_{ij}$ is the interaction effect, and e_{ijk} is the random error.

3. Results

The semen characteristics of Ross 308 broiler breeder males were significantly influenced by the interaction between age and body weight (Table 1).

The interaction between age and body weight had a significant impact on semen volume and individual sperm motility ($P < 0.05$), while age had no significant effect on semen color, sperm concentration, gross motility, or sperm viability ($P > 0.05$). The significant interaction indicates that the influence of body weight on motility varies with age. Normal-weight roosters exhibited the highest motility at 25-29 weeks, whereas older birds (30-39 weeks) maintained higher motility even at greater body weights.

Table 1 - The effects of the interactions between age and body weight on Ross broiler semen characteristics

	25-29 weeks			30-39 weeks			SEM	P-value Age × Weight
	Normal	Underweight	Overweight	Normal	Underweight	Overweight		
Colour (score)	3.00	2.71	2.50	1.78	2.33	3.00	0.72	0.27
Volume (mL)	1.55ab	0.60a	0.70a	0.10a	0.85ab	2.35b	0.51	0.04
Concentration (10 ⁹ /mL)	1.35b	0.57ab	0.74ab	0.22a	0.7ab	1.13ab	0.38	0.07
Gross motility (score)	4.50	2.17	2.92	4.50	3.50	4.80	1.04	0.37
Individual motility (%)	88.00a	31.67c	27.50c	80.00a	56.67b	84.00a	5.97	0.02
Live (%)	57.70	49.67	56.75	66.00	68.09	64.00	10.50	0.72
Dead (%)	41.40	45.54	42.75	33.50	31.34	35.10	9.09	0.85

Means followed by different letters in the same row differ from each other ($P \leq 0.05$) by the Tukey's test.

At 25-29 weeks, normal-weight roosters produced the highest semen volume (1.55 mL), whereas underweight and overweight birds had lower volumes. However, at 30-39 weeks, overweight roosters showed a significant increase in semen volume (2.35 mL), while normal-weight and underweight birds produced significantly lower volumes (0.10 mL and 0.85 mL, respectively). Overweight roosters at 30-39 weeks had a 51.6% increase in semen volume compared with their volume at 25-29 weeks. In contrast, semen volume in normal-weight roosters decreased by 93.5%, from 1.55 mL to 0.10 mL, with age progression.

A significant interaction effect was also observed for individual sperm motility ($P < 0.05$). At 25-29 weeks, normal-weight roosters exhibited the highest motility (88.00%), whereas both underweight (31.67%) and overweight (27.50%) birds had significantly lower values. This represents a 64.0% and 68.8% reduction, respectively, compared with normal-weight roosters at the same age. By 30-39 weeks, overweight and normal-weight roosters showed the highest motility (84.00% and 80.00%, respectively), while underweight birds continued to display lower motility (56.67%). Motility in underweight birds was 32.6% lower than that of overweight birds within the same age group.

Although sperm viability and mortality were not significantly influenced by the interaction ($P > 0.05$), the percentage of live spermatozoa was slightly higher in normal-weight and underweight roosters at 30-39 weeks (66.00% and 68.09%, respectively).

Individual sperm motility was significantly higher in older roosters (30-39 weeks) (69.33%) than in younger roosters (25-29 weeks) (41.26%) ($P < 0.05$) (Table 2). This represents a 67.9% increase in motility with advancing age.

Age did not significantly influence other semen parameters, such as semen color, volume, sperm concentration, gross motility, and viability ($P > 0.05$). However, younger roosters showed a higher percentage of live sperm (66.03%) than older birds (54.71%).

Table 2 - The effects of age on Ross broiler semen characteristics

	25-29 weeks	30-39 weeks	SEM	P-value
Colour (score)	2.74	2.29	0.52	0.38
Volume (mL)	0.85	0.83	0.26	0.67
Concentration (10 ⁹ /mL)	0.89	0.61	0.15	0.42
Gross motility (score)	3.96	3.19	1.08	0.17
Individual motility (%)	41.26b	69.33a	35.64	0.01
Live (%)	66.03	54.71	110.18	0.11
Dead (%)	43.23	33.31	82.63	0.11

Means followed by different letters in the same row differ from each other ($P \leq 0.05$) by the Tukey's test.

Semen parameters, including sperm motility, were strongly impacted by body weight ($P < 0.05$). Normal-weight roosters showed the highest motility (84.00%), while underweight and overweight had significantly reduced motility (44.17% and 46.33%, respectively) (Table 3). Motility in underweight roosters was reduced by 47.4%, and in overweight roosters by 44.9%, compared with normal-weight roosters.

Body weight did not alter semen color, volume, sperm concentration, mass motility, viability, or mortality ($P > 0.05$). Overweight roosters had a semen volume of 1.25 mL, whereas normal-weight birds had 0.58 mL, although the difference was not significant ($P > 0.05$). Sperm concentration was not significantly different across groups, with overweight roosters showing slightly higher concentrations (0.87×10^9 sperm/mL) than normal-weight (0.79×10^9 sperm/mL) and underweight birds (0.64×10^9 sperm/mL).

Table 3 - The effects of weight on Ross broiler semen characteristics

	Normal	Underweight	Overweight	SEM	P-value
Colour (score)	1.25	2.48	2.75	0.52	0.76
Volume (mL)	0.58	0.75	1.25	0.26	0.20
Concentration (10^9 /mL)	0.79	0.64	0.87	0.15	0.60
Gross motility (score)	4.50	3.27	3.87	1.08	0.34
Individual motility (%)	84.00a	44.17b	46.33b	35.63	0.01
Live (%)	61.85	58.8	60.37	110.17	0.92
Dead (%)	37.45	38.44	38.94	82.63	0.97

Means followed by different letters in the same row differ from each other ($P \leq 0.05$) by the Tukey's test.

4. Discussion

4.1. Effect of age on semen characteristics

Sperm motility increased with age in Ross 308 roosters, presumably due to the progressive maturation of the reproductive organs and more effective endocrine regulation (Ansari, 2024). As poultry matures, the development of testicles and androgen synthesis, particularly testosterone, reach optimal physiological levels, thereby increasing spermatogenesis and energy metabolism in spermatozoa (Faure et al., 2017).

Roosters aged 30-39 weeks exhibited significantly higher individual sperm motility (69.33%) compared with younger roosters aged 25-29 weeks (41.26%), representing a 67.9% increase. This suggests that sperm motility improves with age, possibly due to the progressive maturation of the reproductive system and hormonal regulation (Barbarestani et al., 2025; Shaheen et al., 2023). Testosterone levels in broiler breeder males tend to stabilize as they age, which may contribute to enhanced sperm motility and fertilization potential (Khan, 2011; Xue et al., 2022).

The little decline in sperm viability seen in older roosters may be related to the accumulation of oxidative stress over an extended period, resulting in degradation of sperm membranes and DNA (Gao et al., 2021). The reason for this is that, despite the improved motility, the overall viability may not improve substantially with age.

However, additional characteristics of the sperm, such as the colour of the sperm, its volume, the concentration of sperm, and its general motility, were not significantly changed by age. Although there was a decrease in sperm concentration, the variability was not statistically significant. The

concentration of sperm decreased from 0.89×10^9 sperm/mL at 25-29 weeks to 0.61×10^9 sperm/mL at 30-39 weeks from the previous value. Despite the fact that sperm motility improves with age, sperm production levels remain usually stable, as evidenced by the fact that sperm volume remained consistent across all age groups (Bazyar et al., 2019).

Sperm viability was marginally greater in younger roosters (66.03%) compared with older roosters (54.71%), although this difference lacked statistical significance. The reduction in sperm viability with advancing age may be ascribed to cumulative oxidative stress and cellular senescence, which can affect sperm membrane integrity and overall semen quality (Khalil-Khalili et al., 2021; Rasool et al., 2025). The negligible fluctuation indicates that age alone does not significantly affect sperm survival.

4.2. Effect of body weight on semen characteristics

Optimal body weight is crucial for sustaining effective sperm motility in male broiler breeders. Normal-weight roosters presumably possess an optimal balance of metabolic energy and hormonal equilibrium that facilitates testicular function, but departures from the normal weight whether underweight or overweight can impair these physiological processes (Jarrell et al., 2020).

The data indicate that body weight significantly influenced individual sperm motility (Table 3). Normal-weight roosters exhibited the highest sperm motility (84.00%), while underweight (44.17%) and overweight (46.33%) birds had significantly lower motility. This suggests that deviations from the optimal body weight negatively impact sperm movement, which is a crucial factor in fertility (Feyisa et al., 2017). Underweight birds may encounter dietary deficiencies that restrict ATP production, decreasing sperm motility (Gonçalves et al., 2015), whereas overweight birds are susceptible to excessive adipose tissue and related endocrine disruptions, impairing testicular function and elevating oxidative stress (Walzem and Chen, 2014).

The reduced motility observed in underweight roosters may be attributed to nutritional deficiencies that affect ATP production and sperm metabolism, leading to lower motility (Olubowale, 2013; Díaz Ruiz, 2024). Conversely, overweight roosters may experience testicular dysfunction, hormonal imbalances, and increased oxidative stress, all of which contribute to reduced sperm motility (Ansari, 2024).

The negligible impact of body weight on sperm viability and mortality indicates that weight predominantly influences sperm motility rather than overall sperm survival. The reduction in sperm motility in both underweight and overweight roosters indicates that sustaining an ideal body weight is essential for optimizing reproductive effectiveness (Sweeney, 2022).

4.3. Effect of the interaction between age and body weight

The relationship between age and body weight indicates that physiological compensations may arise with ageing. Although younger birds derive more advantages from sustaining an optimal body weight, older birds may exhibit enhanced semen parameters even at elevated weights due to maturity-associated hormonal and metabolic adaptations (Naik et al., 2015).

The interaction of age and body weight had a significant impact on semen volume and individual sperm motility. At 25-29 weeks, normal-weight roosters exhibited superior semen volume and motility compared with both underweight and overweight birds, confirming that deviation from optimal weight in either direction reduces reproductive performance (Behnamifar et al., 2025). However, by 30-39 weeks, overweight roosters exhibited a notable increase in semen volume (2.35 mL), whereas normal-weight (0.10 mL) and underweight (0.85 mL) birds had significantly lower volumes. This indicates that weight gain in older roosters may be associated with increased semen production, though excessive weight gain may still impair sperm motility (Feyisa et al., 2017). Nonetheless, excessive weight adversely affected motility in younger individuals, highlighting the necessity of customised feeding regimens that correspond with reproductive stage and age.

A similar pattern was observed for individual sperm motility, where normal-weight roosters at 25-29 weeks had the highest motility (88.00%), while underweight (31.67%) and overweight (27.50%) birds had significantly lower motility. However, by 30-39 weeks, overweight roosters showed the highest motility (84.00%), whereas underweight roosters continued to exhibit reduced motility (56.67%). This suggests that while normal weight is beneficial for semen quality in younger birds, overweight birds may compensate for reduced motility as they age (Sweeney, 2022).

This study was limited to semen quality parameters and did not include *in vivo* fertility or perivitelline membrane binding assays. However, sperm motility is a well-established indicator of fertilization potential in broiler breeders (Sayed et al., 2022). Future studies should validate these results through fertility or hatchability assessments.

5. Conclusions

This study illustrates that the quality of semen in Ross 308 broiler breeder males is substantially influenced by both age and body weight, with a particular emphasis on sperm motility. Older birds generally demonstrated enhanced motility, irrespective of their weight, while normal-weight roosters demonstrated superior motility compared with underweight and overweight birds. These results underscore the significance of age- and weight-specific management strategies to optimize reproductive performance in broiler breeders. Nevertheless, the study is constrained by the absence of hormonal and seasonal profiling, as well as the relatively small sample size, which may also affect semen characteristics. These factors should be considered in future research to enhance and generalize the results.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Author contributions

Conceptualization: Rahman, M. M. and Izuddin, W. I. **Formal analysis:** Kalam, M. A. **Funding acquisition:** Bhuiyan, M. S. A. **Investigation:** Bhuiyan, M. S. A.; Napoleon, N.; Zailan, M. Z.; Sidik, S. A.; Rasid, R. A. and Yaakub, N. M. **Methodology:** Rahman, M. M. and Izuddin, W. I. **Project administration:** Candyrine, S. C. L. **Supervision:** Candyrine, S. C. L. **Validation:** Rahman, M. M. **Writing – original draft:** Kalam, M. A. **Writing – review & editing:** Kalam, M. A.

Conflict of interest

The authors declare no conflict of interest.

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