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ANIMAL PRODUCTION

Impact of body weight at 19 weeks of Embrapa 051 layers on performance, nest utilization, and egg quality throughout the laying cycle

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ABSTRACT: A total of 860 Embrapa 051 pullets were allocated into three groups based on their 19-week body weights (heavy: 1.48 kg ± 0.01 SD, N= 172 birds, medium: 1.32 kg ± 0.039 SD, N= 516 birds, light: 1.19 kg ± 0.019 SD, N=172 birds) and housed in floor pens with nests for a 65-week production period. Heavy-weight birds exhibited higher egg production during weeks 24-28, while medium-weight hens surpassed heavy-weight counterparts between weeks 41-46, and light-weight hens outperformed heavy-weight birds in weeks 61-65. From weeks 57-65, no significant egg production differences were noted between heavy and medium-weight hens. Body weight at 19 weeks affected floor eggs, cracked eggs, and double-yolk eggs ($P < 0.05$) during weeks 22-34. Heavy-weight hens laid fewer floor eggs, medium-weight hens had fewer cracked eggs (0.3%), and light-weight hens produced fewer double-yolk eggs (1.1%). No significant impact of weight grouping on egg weight was observed. Results indicated that body weight at 19 weeks influences the laying cycle, with heavy, medium, and light-weight hens exhibiting distinct egg production patterns, nest use and egg quality traits at different phases of the cycle.
Key words: cracked eggs, double-yolk eggs, egg production, floor eggs, laying rate, nests.

Impacto do peso corporal às 19 semanas nas poedeiras Embrapa 051 sobre desempenho, uso dos ninhos e qualidade dos ovos ao longo do ciclo de postura

RESUMO: Oitocentas e sessenta frangas Embrapa 051 foram alocadas em três grupos de peso corporal às 19 semanas (pesadas: 1,48 kg ± 0,01 DP, N= 172 aves, médias: 1,32 kg ± 0,039 DP, N= 516 aves, leves: 1,19 kg ± 0,019 DP, N=172 aves) e alojadas em piso com ninhos por 65 semanas. As aves pesadas apresentaram maior produção de ovos nas semanas 24-28, enquanto as médias superaram as pesadas nas semanas 41-46, e as leves superaram as pesadas nas semanas 61-65. Nas semanas 57 a 65, não houve diferenças significativas na produção de ovos entre as aves pesadas e médias. O peso às 19 semanas afetou ovos no chão, ovos trincados e ovos duplos ($P < 0,05$) nas semanas 22-34. As aves pesadas botaram menos ovos no chão, as médias tiveram menos ovos trincados (0,3%), e as leves produziram menos ovos com duas gemas (1,1%). Não houve impacto significativo do peso inicial no peso dos ovos. Em conclusão, o peso às 19 semanas influencia o ciclo de postura, com aves pesadas, médias e leves exibindo padrões distintos de produção de ovos, uso do ninho e características de qualidade em diferentes fases do ciclo.
Palavras-chave: ovos trincados, ovos gema dupla, produção de ovos, ovos de cama, taxa de postura, ninhos.

INTRODUCTION

In the poultry industry, various commercial strains such as Lohmann, Hy-Line, Babcok, Isa, Dekalb, Bovans, Shaver, and Hisex, in their White and Brown variants, can be found for the production of white and brown-shelled eggs, respectively. Each of these strains has its specific characteristics in terms of body conformation, feed consumption, and egg production.

The Brazilian Agricultural Research Corporation (Embrapa) for Swine and Poultry developed the hybrid strain Embrapa 051 by crossing birds from the Rhode Island Red and Plymouth Rock White lines. It was specifically designed to produce brown-shelled eggs. Moreover, these hens are recognized for their resilience and ability to easily adapt to less intensive rearing systems (SOUZA et al., 2011).

According to FORGIARINI et al. (2022), Embrapa 051 strain (E051) stands out as the sole colonial laying hen developed and provided by an official research agency in Brazil. The primary beneficiaries of this specific strain are small family farmers with limited technological resources, managing flocks of 20–25 hens each. These farmers experience increased productivity by replacing native (unselected) hens, weighing approximately 1.8 kg and producing an average of 80 eggs per hen cycle, with E051 hens. The E051 hens can weigh up to 2.4 kg at 90 weeks of age (accepted slaughter weight) and

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yield up to 345 eggs per hen in a 20-90-week cycle (LEDUR et al., 2011; ÁVILA et al., 2019).

Controlling the body weight of pullets according to the management guidelines of each strain is crucial to prevent premature sexual maturity and early onset of egg production. To achieve this goal, it is essential to implement appropriate management programs, including monitoring body weight throughout the entire rearing period (ÁVILA et al., 2007).

Body composition and live weight are significant factors, as laying hens at peak production utilize body tissues and regulate feed consumption based on their body condition (LEESON & SUMMERS, 1987). Maintaining uniform batches is directly related to management and feeding practices, and any alterations in these aspects can affect their productive performance (OLIVEIRA et al., 2001). PÉREZ-BONILLA et al. (2012) asserted that body weight at the onset of laying can be considered one of the main factors influencing hen productivity during the laying cycle. However, there is a shortage of information available in the literature, especially regarding alternative and colonial strains in laying poultry farming. For this reason, the present study aimed to evaluate the effect of three categories of body weight in Embrapa 051 laying hens at 19 weeks of age on productive aspects, nest use, and egg quality during the laying cycle.

MATERIALS AND METHODS

Location, birds, facilities, and treatments

The study was conducted at a free-range egg production and classification unit on the Gross Family Farm, located in the municipality of Ouro, Santa Catarina (Latitude: -27º20'57.05" Longitude: -51º41'40.70"). The research was carried out in collaboration with Embrapa Suínos e Aves, in the municipality of Concórdia, Santa Catarina.

A total of 860 laying hens of the Embrapa 051 hybrid colonial strain (E051) were used, acquired from a specialized rearing company at 110 days of age with an average weight of 947.5g. The birds were housed in the laying house at 16 weeks of age and received a pre-laying diet until 20 weeks of age. At 19 weeks, all birds were weighed and separated into three weight categories, considered as treatments in this study: T1) Light hens (average weight 1.190 kg ± 0.019 SD, N=172 birds), T2) Medium hens (average weight 1.320 kg ± 0.039 SD, N= 516 birds), and T3) Heavy hens (average weight 1480 kg ± 0.01 SD, N= 172 birds).

The birds were distributed into 20 pens (four pens for Light hens, four pens for Heavy hens, and 12 pens for Medium hens), each containing 43 hens, at a density of 7 birds/m^2, and maintained in a cage-free system on a pine shavings bed. All pens were equipped with a tubular feeder, five nipple-type drinkers, and two perches positioned at 25 and 60 centimeters above the ground.

The nests consisted of a black pine board structure measuring 0.90 m in length, 33 cm in depth, and 1.10 m in height, positioned 25 cm above the ground. Each structure contained six nest openings with dimensions of 28 cm in length, 30 cm in width, and 33 cm in height.

The experimental period covered the laying phase from 20 to 65 weeks of age. During this period, all birds were fed with corn and soybean meal-based diet with a minimum nutritional composition established to meet the maintenance and egg production requirements of the Embrapa 051 strain. Table 1 presents the composition of the control diet used during the bird's production phase.

Productive performance and egg quality

The laying rate, measured as a percentage (%), was calculated from the weekly egg production divided by the number of birds in the box. Once a week, all eggs produced on that day were individually weighed using a high-precision digital scale (0.01 g) (Unishow®, China) to calculate average egg weights. Throughout each day, eggs were collected at four distinct times: 09:00, 11:00, 14:00, and 17:00. For each collection time, the total number of eggs produced and their location (in the nest or on the floor) were recorded. Additionally, an experienced observer classified the eggs through candling, identifying if they were normal, cracked, deformed, or had double yolks.

Statistical analysis

Statistical analyses were performed using version 4.2.1 of the R software (R CORE TEAM, 2022) and Microsoft Excel 2019 (Microsoft Corporation, Redmond, WA, USA). To calculate 95% confidence intervals (CI) for the means of bird performance variables, the Wald approximation was used. Furthermore, linear models were adjusted to estimate the percentage of egg production and egg weight, considering the weight of the birds at the beginning of the laying cycle and the age of the birds in weeks as predictor variables. The following model was used: $Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + E_{ijk}$, where Y_{ijk} is the response variable, μ is the overall mean, A_i is the simple effect of the weight of the birds

Table 1 - Ingredients and nutritional composition of the diet.

Ingredients	Kg
Whole Corn	60.0000
Soybean Meal	20.2958
Calcitic Limestone	8.9094
Wheat Bran	6.4114
Soybean Oil	2.7570
Dicalcium Phosphate	0.6902
Iodized Salt	0.3996
[1]Premix	0.3000
DL-Methionine	0.1049
[2]Mycotoxin Sequence	0.1000
L-Lysine HCl	0.0186
BHT (Butylated Hydroxytoluene)	0.0100
[3]Phytase	0.0030
Total	100.00
Nutritional Composition Calculated	Mean
Metabolizable Energy (EM) kcal/kg	2800
Crude Protein (PB), %	15.00
Digestible Phosphorus (Pdisp), %	0.350
Calcium (Ca), %	3.700
Sodium (Na), %	0.170
Chloride (Cl), %	0.170
Linoleic Acid, %	1.500
Choline, mg/kg	1130
Digestible Lysine, %	0.680
Digestible Methionine, %	0.340
Digestible Methionine + Cysteine, %	0.610
Digestible Threonine, %	0.490
Digestible Tryptophan, %	0.150
Digestible Arginine, %	0.690
Digestible Isoleucine, %	0.550
Digestible Valine, %	0.620

[1]Ovotec Matrix P-3®: Minimum composition per kilogram of product: Copper: 3330mg; Iron: 16.65g; Manganese: 33g; Selenium: 100mg; Zinc: 33.3g; Vitamin A: 4000800IU; Vitamin D3: 1000200IU; Vitamin E: 30000IU; Vitamin K3: 1674mg; Vitamin B1: 980.2mg; Vitamin B2: 4000mg; Vitamin B6: 1633.7mg; Vitamin B12: 10000mcg; Folic Acid: 1060mg; Pantothenic Acid: 4980mg; Niacin: 16g; Biotin: 100mg; Choline: 140.6g; Iodine: 660mg; [2]MastersorbGold®; [3]Natuphos®: 10000 FTU.

at the beginning of the laying cycle, B_j is the age of the birds and $(AB)_{ij}$ is the interaction of initial body weight and age of the birds, and E_{ijk} is the random error. To check the model assumptions, the following tests were conducted: Shapiro-Wilk normality test to assess the normality of residuals, Levene's test to evaluate the homogeneity of variances, and Breusch-Pagan test to assess heteroscedasticity (homogeneity of error variance). After model adjustment, all treatments were compared using estimated marginal means (emmeans), employing the Tukey test as a correction factor for multiple comparisons of means. Associations between egg laying frequencies in the nest or on the floor, cracked eggs, double-yolk eggs, and deformed eggs during the 22-34 week period with the weight of birds at the beginning of the laying cycle were tested using the Pearson chi-square test. P-values < 0.05 were considered statistically significant in all analyses conducted.

RESULTS AND DISCUSSION

Laying rate

The adjusted linear model to estimate the percentage of egg production with the classification of hens according to initial weight, age of the birds in weeks, and the interaction between these factors as predictor variables explained a statistically significant and substantial proportion of the variance ($R^2 = 0.97$, $P < 0.001$). As can be seen in figure 1, the egg laying of heavy birds started in the 23rd week and reached the peak of production in the 29th week with 87.1% laying (CI 82.4% to 91.81%). Birds in the light and medium categories reached the peak of production in the 32nd week with 86.4% (CI 81.7% to 91.2%) and 85.8% (CI 83.1% to 88.5%) laying, respectively, maintaining production above 70% until 61 weeks of age. Significant differences ($P \leq 0.05$) were found between categories during weeks 24 to 28 of the laying cycle, with heavy layers achieving a higher laying rate than medium and light birds (Figure 1).

Between weeks 29 and 40, no significant differences in laying rates based on bird weight were observed. However, from weeks 41 to 46, medium-weight hens exhibited a higher laying rate ($P \leq 0.05$) compared to heavy hens, aligning with the rates of light hens. Towards the end of the cycle (weeks 61 to 65), light hens showed a higher laying rate ($P \leq 0.05$) compared to heavy hens, similar to medium-weight hens.

The initial weight of birds at the beginning of the laying cycle impacts their laying rate throughout the cycle due to its effect on their overall health, energy reserves, and ability to maintain egg production. Heavier pullets are preferred, as anecdotally they seem to be more resilient during the transition from rearing to laying and tend to produce larger eggs earlier in the laying period (MUIR et al., 2023). Studies on selecting for live weight gain in laying hens have shown that increased body weight results in decreased egg production, larger eggs, and higher feed consumption, as heavier birds consume more feed and produce larger eggs with larger yolks compared to lighter hens, highlighting the importance of weight control (LACIN et al., 2008).

SUN & COON (2005) reported similar findings, indicating heavier pullets laid earlier and reached 50% production sooner. Nonetheless, light birds produced as many eggs as medium and heavy breeder hens from weeks 20 to 65.

According to ÁVILA et al. (2017), Embrapa 051 hybrid hens peak at 27 to 29 weeks with 90-91% production, decreasing linearly to 68% at 65 weeks. This study noted delayed sexual maturity in all weight categories compared to Embrapa 051 guidelines at 19 weeks. Despite heavy hens starting laying at 23 weeks, they had lower production (61%) at 65 weeks compared to light (65%) and medium (67%) hens, with a more pronounced decline.

Factors influencing sexual maturity include age, nutrition, and photoperiod (ARTONI et al., 2019). Weight and age play roles, with a threshold between weight and ovulation onset. The study found no statistically significant differences ($P > 0.05$) in average egg production (23 to 65 weeks) among heavy (71.3%), medium (73.6%), and light (72.1%) categories, in agreement with OKPOKHO et al. (1987).

LARA et al. (2019) recommended a 10% weight variability to avoid adverse effects. Heavier hens may impact profitability due to higher feed consumption, without a proportional increase

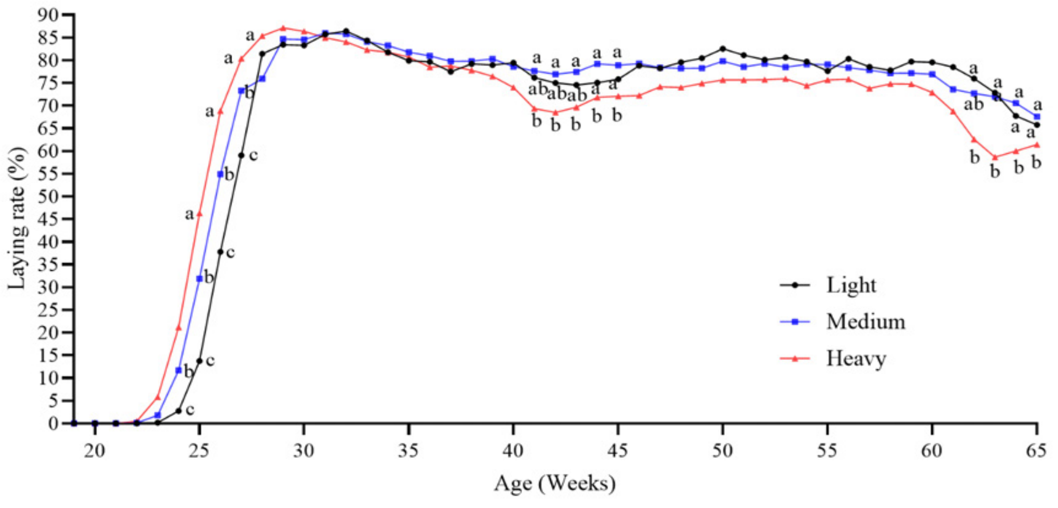

Figure 1 - Effect of bird weight at 19 weeks of age on egg production rate throughout the laying cycle (20 to 65 weeks) of Embrapa 051 layers.

in egg production. Lighter hens may experience delayed maturity, lower production, and reduced peak production.

Nest use

Nest box use is important in laying hens, and gregarious nesting, which involves choosing an occupied nest site, is associated with welfare problems and increased risk of broken or dirty eggs (RIBER, 2010). The effect of the birds' initial weight on the number of eggs laid in nests or on the floor was evaluated from the 22nd to the 34th week of the birds' life and at four collection times (9 AM, 11 AM, 2 PM, and 5 PM).

As observed in figure 2, the quantity of eggs laid outside the nest was affected by the birds' initial weight. During the 11 AM collection period, heavy category hens significantly laid fewer eggs on the floor (3.1% - 92/2941) compared to medium (5.2% - 481/9180) and light category hens (5.8% - 170/2928).

In the 2 PM collection period, similar behavior was observed to the previous collection time, where heavy category hens significantly laid fewer eggs on the floor (3.3%, 40/1208) compared to medium (5.4% - 218/4024) and light category hens (6.0% - 85/1418).

In cage-free systems, one of the major concerns is the location where hens lay eggs, as floor-laid eggs can cause economic losses and food safety issues (DE REU et al., 2008; JONES et al., 2015).

The use of nests by hens depends on various factors, including nest density, light intensity inside the nest, nest color, the presence of eggs in the nest, nest bedding material, nest type and shape, and the position and location of the nest. Exposure to these factors during the rearing phase influences the experience and nest usage preferences during the laying cycle (CLAUSEN & RIBER, 2012).

To ensure proper nest utilization, in addition to adequate size, it is recommended that there be a difference in light intensity inside the nest

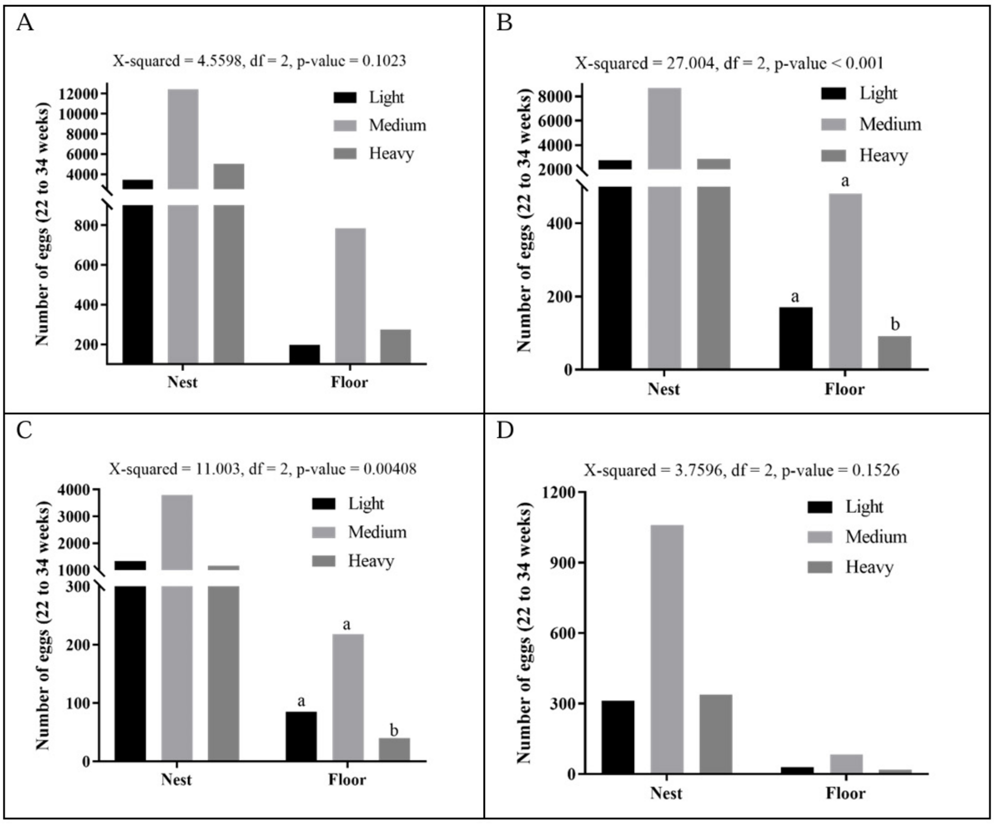

Figure 2 - Effect of initial weight of Embrapa 051 layers on the number of eggs laid in the nest or on the floor at A) 9 AM, B) 11 AM, C) 2 PM, and D) 5 PM, from 22 to 34 weeks of age.

(darker inside) to make the birds feel sheltered (BIST et al., 2023). The nest should also be large enough for the hens to turn their bodies and scratch with their feet to complete the egg-laying behavior (DUNCAN & KITE, 1989).

Another crucial factor is nest density, i.e., the number of hens that need to share the same nest during egg-laying. It is often observed that several hens use the same nest simultaneously. Hens often choose already occupied nests for egg-laying, even if other nests are unoccupied. Moreover, egg-laying behavior in laying hens is influenced by the environment they live in (CLAUSEN & RIBER, 2012).

In this experiment, the nest density was seven hens in all weight categories, so this factor alone does not explain the lower percentage of floor-laid eggs in heavy category hens. This fact has been confirmed by ABRAHAMSSON & TAUSON (1997), who found no differences in the proportion of eggs laid in or outside the nest when the nest was shared by 5, 6, 7, or 8 hens simultaneously.

There is also strong evidence that the genetic basis of birds significantly influences nest usage rate (ABRAHAMSSON et al., 1996).

In this experiment, it was confirmed that Embrapa 051 laying hens are highly motivated to use nests due to the high percentage, on average above 95%, of eggs laid in this accessory.

Conversely, it is true that nest usage increases with age (APPLEBY et al., 1993; ALVEY et al., 1996). To prevent stress and possible egg-laying outside the nest, it is important to design nests so that hens can perform pre-laying and egg-laying behaviors in an appropriate location, minimizing competition (DUNCAN & KITE, 1989; COOPER & APPLEBY, 1995).

Furthermore, it is necessary to ensure that nests are managed correctly to keep eggshells clean and intact, ensuring an ideal production system (VILLANUEVA et al., 2017).

The hypothesis in this study was that egg-laying, whether in the nest or on the floor, may be influenced by the weight of laying hens. The body weight of laying hens is directly related to their nutritional status and health.

ALDRICH & RAVELING (1983) found that heavier females spent more time in nests incubating eggs and lost more weight during incubation than lighter females in captive Canada geese. The reason for this could be that lighter females needed to leave the nests more often to forage for food to maintain their lipid reserves. In this study, lighter category hens might be more motivated to search for food to increase their body reserves than heavy category hens, and for this reason, they used the nest less for egg-laying.

Additionally, OLIVEIRA et al. (2019) observed a lower number of floor-laid eggs in heavy category hens, possibly due to sexual maturity, as they started laying before the classes of lighter and medium-weight birds and thus learned better nest usage. In this study, it was observed that heavy category birds started laying eggs before medium and light categories, which could help explain the higher quantity of eggs placed in nests in this weight category.

Regarding the egg-laying time, the observed results are similar to those of HUNNIFORD et al. (2017), where birds laid most eggs 3.5 to 4.5 hours after the lights were turned on in the aviary.

Egg quality

Egg quality throughout the laying cycle was evaluated based on egg weight, normal, cracked, double-yolked, and deformed eggs, considering the initial body weight category of the birds. Producing high-quality eggs reduces waste and increases the overall profitability of the egg production operation. Poor-quality eggs result in higher losses and lower returns, impacting the economic sustainability of the farm.

No significant effect ($P \geq 0.05$) of the birds' initial weight on egg weight during the laying cycle was observed. As the birds aged, all weight categories of hens showed a linear increase in egg weight, ranging from 52 grams at 30 weeks of age to 60 grams at 65 weeks of age.

DU PLESSIS & ERASMUS (1972), studying South African White Leghorn hens with body weights ranging from 1.36 to 2.27 kg, found that the positive correlation between body weight, egg production, and egg weight becomes antagonistic when birds weigh more than 2.27 kg. Therefore, the authors recommend discarding young birds that reach this weight at sexual maturity for egg production. The same authors also observed that hens categorized as light not only have low egg production but also produce eggs with weights below the recommended values.

According to FARIA et al. (2019), the distribution of egg weight throughout the bird's productive life is a crucial factor, especially in genetic improvement. According to the authors and the results observed in this study, egg weight increases with age, depicting two situations: smaller eggs at the beginning of the production phase and larger eggs in the final third of the productive life, which may lead to shell quality issues.

AKANBI & GOODMAN (1982) found that egg weights in medium and heavy weight bird categories were higher (P < 0.05) than in lighter birds. This significant difference was observed during the early stages of laying and generally persisted throughout the experiment. SUMMERS & LEESON (1983) state that body weight appears to be the main factor influencing the initial egg size, supporting the findings of PÉREZ-BONILLA et al. (2012) and OKPOKHO et al. (1987), who also observed that heavier hens produce heavier eggs during the laying cycle.

Deformed, cracked, and double-yolk eggs

The evaluation of the production of normal, cracked, double-yolk, or deformed eggs during the period from 22 to 34 weeks of age is presented in figure 3.

The quantity of cracked eggs was affected by the initial weight of the hens. It can be observed that medium-weight hens had a significantly lower percentage of cracked eggs (0.3% - 82/27662) compared to heavy (0.5% - 45/9859) and light-weight hens (0.5% - 40/8404).

In figure 3B, it can be observed that the quantity of double-yolk eggs was also affected by the initial weight of the hens. Light-weight hens had a significantly lower quantity of double-yolk eggs (1.1% - 96/8460) compared to medium (1.6% - 447/28027) and heavy-weight hens (1.5% - 148/9962). The number of deformed eggs was not significantly affected by the weight categories evaluated (Figure 3C).

Studies related to egg quality have been conducted due to their direct connection with commercialization. Therefore, evaluating factors that affect egg quality is of utmost importance for consumer acceptance. One such factor is shell integrity, which depends on adequate bird nutrition,

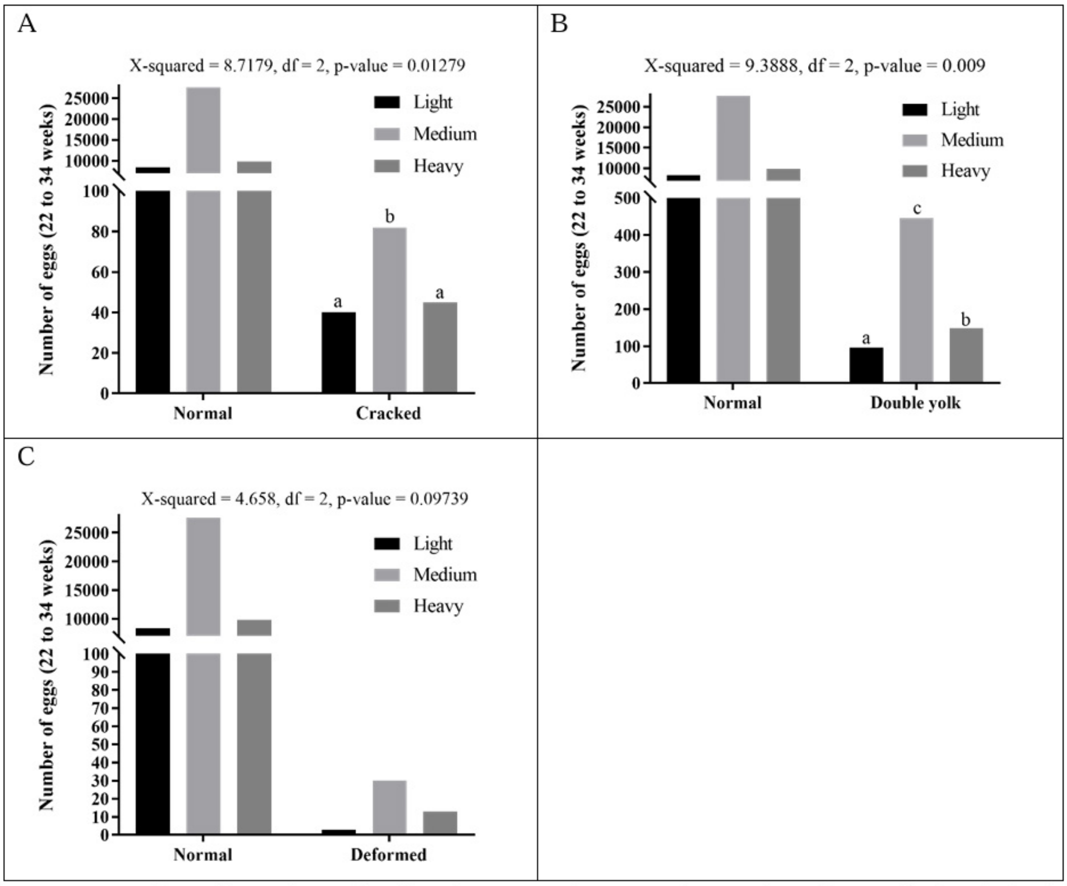

Figure 3 - Effect of initial weight of Embrapa 051 layers on the number of eggs A) Cracked, B) Double-Yolk, and C) Deformed, from 22 to 34 weeks of age. Idem 1.

including a proper balance of minerals, especially calcium and phosphorus, as well as electrolyte balance for homeostasis maintenance. Additionally, care is required during egg handling, transport, and storage (BAPTISTA et al., 2007). In the conducted study, a higher number of cracked eggs was observed in medium-weight category hens, possibly due to a higher incidence of floor laying. Besides the contamination problem, this can result in cracks in the eggshells.

LEESON & SUMMERS (1987) conducted two experiments with Leghorn breed birds, dividing them into three weight categories: light, medium, and heavy. The first experiment was conducted when the birds were 15 weeks old, while the second occurred when they were 19 weeks old. In Experiment 1, body weight had no impact on eggshell deformation. However, in Experiment 2, medium-weight birds produced eggs with superior shell quality. Conversely, BISH et al. (1985) demonstrated that heavy-group hens laid eggs with more shell deformities when evaluating the influence of body weight on egg production.

Laying eggs with double yolks per chicken is considered an anomaly as it represents an irregularity in the hormonal cycle. According to ARTONI et al. (2019), the hen's reproductive system is a highly organized functional structure with the primary goal of forming the yolk (ovarian follicle). This requires the involvement of the central nervous system (hypothalamus), adeno-hypophysis, and the ovary, which interact to promote maturation and release of the follicle in the ovary (yolk). The main stimulators of sexual maturation are age, nutrition, and photoperiod. Therefore, laying eggs with double yolks occurs due to the simultaneous release of two follicles.

One of the factors that can lead to the production of eggs with double yolks is the age of the birds, considered a common occurrence in younger hens as they begin to ovulate, given that the hormonal cycle can still be irregular, resulting in the release of two yolks. MA et al. (2017) also mentioned a higher incidence of double-yolk eggs at the beginning of laying.

Another factor related to this anomaly is linked to genetics. According to WOLC et al. (2012), among defective egg characteristics, the incidence of double-yolk eggs showed a higher heritability estimate ($h2 = 0.27$). The authors observed that double-yolk eggs are positively correlated with egg weight and body weight and negatively correlated with the total number of eggs. They concluded that heavier eggs and a higher body weight of birds are associated with a higher frequency of double yolks and, to a lesser extent, more defects in shell quality.

A positive correlation estimate between double yolks and body weight was reported by ABPLANALP et al. (1987), where the number of eggs with double yolks increased from 2 to more than 30 in 11 generations of selection until 40 weeks of age. The presence of double-yolk eggs was more frequent in hens that produced more eggs with shell defects. Conversely, SUN & COON (2005) claimed not to have found significant differences in the quantity of double-yolk eggs when dividing hens into three different weight categories.

CONCLUSION

The initial weight of the birds has a significant impact on production and quality, including laying rate, nest usage, and the incidence of cracked and double-yolk eggs. These results emphasize the importance of considering initial weight when planning the management and production of Embrapa 051 laying hens. This study did not account for environmental variables and other factors that could influence laying rate and egg quality; however, the findings can be applied to improve weight management strategies in laying hens to enhance egg production and overall flock health.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the design and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

All procedures performed in the study involving animals followed the ethical standards of the Ethics Committee on Animal Experimentation (CEEA) at the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), Concórdia, SC, Brazil, under registration number 13/2022.

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