Relative Effectiveness of Management Techniques: Spiking and Intra-Spiking on Broiler Breeder Production, Hatchability, and Fertility Traits

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Abstract

The objective of the present study was to assess the impact of spiking and intra-spiking on mitigating the decline in fertility and hatchability associated with aging roosters in broiler breeder flocks. A total of 162 Ross-308 strain birds were utilized, categorized into six replicates, each consisting of nine individuals (eight hens and one cock), which were randomly assigned to one of three management treatments: group 1 (control group), group 2 (spiking group), or group 3 (intra-spiking group). Data were systematically collected over a period spanning from 42 to 62 weeks of age. Semen samples were obtained four times during the experimental duration, with subsequent evaluations of semen characteristics. Additionally, blood samples were procured for quantifying concentrations of insulin, creatinine, testosterone, and nitric oxide. Following the experimental phase, each rooster

from every replicate was weighed and subsequently slaughtered, wherein testicular tissues were harvested for histological analysis. Fertility and hatchability rates were computed based on the collected data. Statistical analysis revealed no significant differences in semen characteristics among the experimental groups. However, the spiking group exhibited a statistically significant increase in testicular weight compared to the control and intra-spiking groups ($p \leq 0.05$). Furthermore, there were greater counts of spermatocytes, spermatids, and spermatozoa in the spiking group ($p \leq 0.05$). Conversely, the control group demonstrated elevated numbers of Sertoli cells, increased seminiferous duct diameters, and greater thickness of the germinal epithelium compared to the treatment groups ($p \leq 0.05$). The decline in fertility and hatchability rates associated with advancing age was less pronounced in the spiking group when compared to the other experimental groups. While concentrations of insulin and creatinine did not exhibit significant variations among the groups, testosterone and nitric oxide levels were significantly higher in the spiking group ($p \leq 0.05$). The research findings indicate that spiking represents an effective strategy for maintaining fertility and enhancing hatchability rates in aged broiler flocks, as well as consequently increasing the economic profitability for broiler breeder producers.

KEYWORDS: Broiler breeder; Spiking; Intra-spiking; Fertility; Hatchability

1. Introduction

The natural decline in fertility among broiler breeder flocks with age poses a threat to economic production (1). Fertility relies on the productive status, interest, and mating capability of the birds (2). As male birds age, mating activity decreases due to higher body weight, lower testosterone levels, and musculoskeletal diseases (3). Fertility typically rises from 23-24 weeks, peaking at 35-37 weeks, before declining between 40-45 weeks, with older

birds showing a more significant reduction (4). To address this decline, various methods, particularly movement within rooster flocks such as intra-spiking and spiking, are employed. Many industries employ spiking and intra-spiking, especially in older broiler breeder flocks. This involves introducing young, unfamiliar males or exchanging experienced males between poultry houses on the same farm. The goal is to enhance fertility levels in established flocks (5).

After reaching 40 weeks of age, breeder hens require more frequent mating to maintain fertility, while rooster mating interest typically declines (1). Additionally, egg hatchability decreases with advancing age (6). To address the fertility decline linked to aging roosters, various strategies focus on movement within rooster flocks, such as intra-spiking and spiking.

The male broiler breeder is crucial for ensuring egg fertility, often more so than the female (7). Mating activity is influenced by male fitness and female receptivity, which can be affected by the sex ratio and feeding practices (8). Introducing intra-spiking has shown potential to mitigate libido decline in aging males, thereby enhancing long-term flock fertility (1,8).

Research indicates that incorporating 24-week-old males into a 45-week-old broiler breeder flock significantly increases hatchability, likely due to the heightened libido of younger roosters. Additionally, females may preferentially mate with younger males exhibiting pronounced secondary sex characteristics (9).

Intra-spiking involves relocating roosters within the same farm, allowing for the replacement of male broiler breeders without introducing external males (10). This practice offers advantages such as cost-effectiveness, reduced disease transmission risk, ease of implementation, and rapid results (11). However, while it may enhance mating activity, fertility can decline after 4-8 weeks due to the similar ages of the males, with a typical fertility increase of 1-3% expected within 5-10 weeks post-spiking (10).

In contrast, a study by Patil and colleagues on the intra-spiking of Vanaraja chickens at 48 weeks revealed only a slight and statistically insignificant increase in fertility rates at 52 and 55 weeks, and it indicated no impact on hatchability. The effectiveness of spiking may vary based on factors such as the age of the breeder flock, spiking frequency, and the ratio of exchanged roosters (4).

Male contribution to flock fertility is influenced by mating activity and sperm quality, both of which decline with age, leading to reduced semen volume and the fertilizing capability of spermatozoa (12).

To the authors' best knowledge, there has yet to be a comprehensive investigation into the effects of spiking and intra-spiking on sperm characteristics, testicular histology, sperm parameters, and various production factors. Therefore, the present study aims to evaluate the effectiveness of male spiking and intra-spiking on reproductive performance in Ross 308 broiler breeder flocks, focusing on fertility, hatchability, testicular histology and sperm characteristics.

2. Materials and methods

2.2. Birds, housing, and management

The research comprised three experiments involving 162 birds across three treatments, each with six replicates. Each replicate consisted of nine birds (8 hens and 1 cock). The birds were randomly assigned to one of following managerial treatments: group 1 (control group, no management), group 2 (spiking group), and group 3(intra-spiking group). The testing phase began when the birds reached 38 weeks of age, and data on experimental treatments were collected for weeks 40 to 62 (post-peak). The environmental conditions at the breeding farm, including moisture content, temperature, weight control, feed intake management, and other parameters, were maintained following the Ross 308 broiler breeder guide (13). The experimental treatments included three management methods: the recommended practices for

broiler breeders of Ross 308, the spiking method (introducing a 28-week-old rooster to the herd), and the intra-spiking method (using roosters of the same age within halls or pens to stimulate early rooster mating activity). The ration composition, based on the nutrition guide for broiler chicken Ross 308 (14), included corn, wheat, soybean meal, oyster powder, calcium phosphate disodium, salt, mineral, and vitamin supplements, as well as methionine and lysine (Table 1). *(Please insert Table 1 near here)*

2.2. Collection and evaluation of semen samples. Before the experiment began, semen was collected from each rooster at least three times to train the male birds (15). Throughout the experiment, semen was collected four times and transferred to the laboratory in a warm water bath. Using an optical microscope, qualitative and quantitative sperm characteristics, including semen volume, sperm density, sperm motility percentage, progressive sperm motility, and the percentage of dead and live sperm, were assessed.

Semen samples were collected using the abdominal rubbing method, which involved stimulating the roosters by rubbing their belly and back on the technician's leg to facilitate ejaculation. The ejaculated semen was collected using 1.5 ml graduated micro tubes. To prevent cold shock to the sperm, the micro tubes were carefully placed inside nylon bags before being transferred to a 37°C warm water flask and transported to the laboratory. Upon arrival at the laboratory, the semen volume was promptly determined using the graduated body of the microtube. For the sperm motility survey, samples were incubated at 37°C for 30 min, followed by dilution in a physiologic serum of one to 200 ml. A drop of diluted sperm was then examined under a microscope at 400x magnification, and the counts of progressive, non-progressive, and immotile sperm were conducted using a graded screen. Each specimen was evaluated for 200 to 400 sperm. Sperm concentration was determined by diluting the sample 1 to 400 ml in distilled water, and subsequently, a drop of the diluted sample was placed on a Neubauer chamber or Hemocytometer for examination under a microscope at

400x magnification. The semen concentration was calculated based on the count obtained from four lateral squares and one central square, using a specific formula for the calculation.

$$C = \frac{(NOSC \times D)}{5(NOS) \times 4(SCS)}$$

Where: C = concentration (nl), NOSC = count of sperm count, D = dilution, NOS = number of sperm counted per square, and SCS = sperm concentration in each home (nl).

To determine the proportion of live and dead spermatozoa in semen samples, a technique involving the application of 10 µl of diluted specimen in distilled water (1:400 ml) on a glass slide, accompanied by the addition of a single drop of Eosin-Nigrosine, was executed. Subsequently, a drop of the resultant solution was spread at 45° on another glass slide. Following desiccation, a microscope magnified 400 was used to enumerate discolored spermatozoa (indicative of dead sperm) and those lacking color (representing viable sperm).

2.3. Determination of testicular characteristics. On day 156 of the experiment, the assessment of testicular characteristics was conducted, which involved randomly selecting and slaughtering a rooster from each nest. Before slaughtering, the roosters, they were weighed and then the testicles were weighed. Subsequently, transverse sections with a maximum thickness of 0.5 cm were carefully prepared from each testicle. The tissue specimens were preserved in 10% formalin solution, followed by slide preparation using established protocols. Subsequently, the quantification of spermatogonia, primary spermatocytes, spermatids, spermatozoa, Sertoli, and Leydig cells was conducted using a 10×10 grid scale graticule. Additionally, the seminiferous duct diameter of and germinal epithelial thickness were measured using a linear graticule.

2.4. Blood hormones and metabolites. Throughout the experiment, blood samples were systematically obtained from roosters at 30-day intervals to assess the levels of creatinine, insulin, testosterone, and nitric oxide. Blood samples were collected from the wing vein and

subsequently centrifuged at 300 rpm for 20 min at a 4°C. The separated serum was then preserved at a temperature of -20°C until biochemical analysis. Subsequently, the samples were dispatched to the biochemical laboratory for thorough analysis.

2.5. Calculation of fertility and hatchability. The calculation of the fertility rate, hatchability rate, and Hatch of Fertility (HF) in chicken production in response to the experimental treatments was carried out using the following formulas:

Fertility rate (%) = (Total number of fertile eggs / Total number of eggs set) × 100

Hatchability rate (%) = (Total number of chicks hatched / Total number of fertile eggs set) × 100

Hatch of Fertility (HF) = (Number of hatched chickens / (number of eggs-unfertilized or infertile eggs)) × 100

2.6. Statistical data analysis

A balanced completely randomized design (CRD) and the MIXED model, which uses repeated measurements over time in SAS software version 9.1, were used for data analysis. The statistical model incorporated the management method, sampling time, interaction between sampling time and management method, and random effects of roosters within the management method. The model structure followed an autoregressive pattern. For data that lacked repetitive patterns over time (testicle weight and histological data), the GLM procedure was used. The Least Squares Means (LSMeans) with a standard error of means (SEM) were used to present the means in the results tables. To compare the means, Duncan's multiple-range test was used. The significance level was considered 5% ($p \leq 0.05$), whereas the trend was discussed at $0.05 \leq p \leq 0.10$.

3. Results

3.1. Qualitative and quantitative of semen characteristics. The results of using management techniques, namely spiking and intra-spiking, for parameters including volume

(ml), concentration ($\times 10^6$), motility, progress motility (%), and viability of spermatozoa are depicted in Figure 1. As illustrated in Figure 1, no statistically significant differences were observed among the experimental groups. *(Please insert Figure 2 near here)*

3.2. Testicular characteristics. The results of applying spiking and intra-spiking management methods on the weight of both testicles and right and left testicular weights are presented in Table 2. There were no significant differences in body weight between the experimental groups. However, the weight of both right and left testicles was higher in the spiking group compared to the intra-spiking group ($p \leq 0.05$). *(Please insert Table 2 near here)*

Table 3 summarizes the histological data of the testicles in response to treatment. Treatments did not significantly affect the numbers of spermatogonia and Leydig cells. In contrast, the spiking management method increased the numbers of spermatocytes, spermatids, and spermatozoa cells compared with the other experimental groups ($p \leq 0.05$). The number of Sertoli cells, seminiferous duct diameter, and germinal epithelium thickness were greater in the control group compared to the two treatment groups ($p \leq 0.05$). *(Please insert Table 3 near here)*

3.3. Fertility and hatchability. Percentage fertility, hatchability rate, and hatch of fertility are shown in Tables 4 and 5. As shown in Table 4, there was no significant effect between management methods on the fertility of total eggs; however, the effect of time on total egg fertility was significant ($p \leq 0.05$). A similar trend was observed in the infertility of total eggs. Table 4 also shows that the percentage of infertile eggs increased with herd age. This was especially true for the control group compared to the other two experimental groups. The rate of reduction in the spiking group is lower compared to the other two groups, although it was not statistically significant.

In Table 5, the percentage of hatchability and hatchability of fertile eggs were not affected by management methods, Of course, the effect of time was significant for both parameters ($p \leq 0.05$). *(Please insert Tables 4 and 5 near here)*

3.4. Blood hormones and metabolites. Figure 2 shows the analysis of blood parameters in response to managerial treatment. *(Please insert Figure 2 near here)*

Applying management treatments, had no significant effect on blood creatinine and insulin concentrations. The testosterone concentration in the first and second sampling times was affected by treatments; thus, in the first sampling time, the control group had a higher testosterone concentration than the two other experimental groups ($p \leq 0.05$). In the second sampling time, the spiking group had a higher testosterone concentration than the other experimental groups ($p \leq 0.05$).

Regarding the trend of changes in the concentration of nitric oxide during the experiment, unlike testosterone, significance was observed between treatments in the last three sampling times. In such a way that in the third, fourth, and fifth sampling times the spiking group had a higher nitric oxide concentration in comparison with two other experimental groups ($p \leq 0.05$).

4. Discussion

The reproductive potential of roosters is significantly influenced by both the quantity and quality of sperm generated in their testicles. In broiler breeder flocks, one rooster typically mates with multiple hens, which underscores the importance of sperm characteristics in determining the overall fertility of the flock. Three primary parameters are commonly utilized to assess sperm fertility: concentration, viability, and mobility. As roosters age, there is a noticeable decline in reproductive performance. This decline manifests as a reduction in semen volume and a decrease in the total number of spermatozoa produced per ejaculation. Additionally, older roosters may exhibit decreased fertilization capacity of their sperm cells, which can adversely impact the overall fertility rates of the hens they mate with (16,17). In the

present study, the application of spiking and intra-spiking management techniques did not significantly affect the characteristics of sperm such as semen volume, sperm concentration, motility, progressive motility, or percentages of alive and dead sperm.

Male broiler chickens exhibiting body weights below 3,800 g are often characterized by infertility or subclinical infertility, which is associated with diminished testosterone levels and elevated corticosterone concentrations. Conversely, heavier male broilers demonstrate larger and healthier testicular structures, alongside increased testosterone concentrations and reduced corticosterone levels. Despite these advantageous physiological traits, heavier roosters encounter significant difficulties in mating due to their size. Factors such as heterogeneity among the male population, the establishment of a pecking order within the flock, and diminished hatch rates serve as impediments to effective mating and access by hens. Furthermore, a decline in reproductive performance in roosters after 45 weeks is correlated with reductions in testicular weight, sperm production, and testosterone levels (16). In the present study, the spiking group exhibited a higher body weight numerically, this difference did not achieve statistical significance. Notably, testicular size was significantly greater in the spiking group compared to the other two groups. The findings align with the work of Fragoso et al., which indicated that increase in testicular size is closely linked to sperm production, as a considerable portion of testicular tissue is devoted to spermatogenesis (16). Furthermore, existing research has demonstrated a positive correlation between age and testicular size, indicating that older roosters tend to possess larger testicles than their younger counterparts (18). An increase in testicular size, along with a more developed network of blood vessels supplying sufficient blood to the testicular tissue, is crucial for optimal spermatogenesis. However, when sperm are not subjected to a suitable environment, their lifespan is diminished both in the seminiferous ducts and the oviduct of chickens (19). Additionally, as roosters age,

there is a noticeable decline in the quality of the testicles, which leads to reductions in both sperm production and testosterone levels (16).

Fertility rates and the number of hatched chicks are essential metrics for evaluating successful reproduction, reflecting the overall efficacy of the mother flock. Infertility may indicate underlying issues within the male population of the herd. Globally, management techniques such as spiking and intra-spiking are frequently employed to mitigate reduced libido in aging roosters (1). In the present study, the spiking method was implemented to demonstrate a reduction in infertility rates and an enhancement in overall egg fertility. However, these changes did not yield statistically significant differences among the experimental groups. Similar patterns were observed in the hatchability of both total and fertile eggs. These findings contrast with those reported by other researchers (8, 20), who noted a significant impact of the spiking method on egg fertility and hatchability, effectively reversing declines in these parameters. Conversely, some studies have indicated that spiking did not significantly affect egg fertility and hatchability (21,22). Fertility is directly linked to the characteristics of the parent flock. As the breeder flock ages, both egg fertility and hatchability tend to decrease, often as a result of reduced mating frequency, lower sperm motility, and decreased sperm penetration into the egg in aging broiler breeders (17).

The concentrations of blood creatinine and insulin did not demonstrate statistically significant differences; however, testosterone levels exhibited a declining trend in relation to the increasing age of the roosters throughout the experimental period, regardless of treatment conditions. Specifically, the spiking group displayed significantly higher testosterone concentrations during the interval between 40 and 50 weeks of age when compared to the other groups. Additionally, a notable distinction in nitric oxide concentration was observed in the spiking group between 50 and 60 weeks of age relative to the other groups. Testosterone is a critical hormone influencing age-related changes in fertility, affecting various

physiological traits such as testicular development, modulation of Sertoli cells, as well as sexual behavior and mating activity (20). The finding of this study align with previous research has established a positive correlation between sexual behavior and testosterone levels in roosters (4). Furthermore, older male birds exhibit reduced testicular responsiveness to luteinizing hormone (LH) concerning testosterone production compared to their younger counterparts (23).

Nitric oxide, functioning as both an intra- and intercellular signaling molecule, plays a pivotal role in the regulation of hormone secretion. It exerts autocrine and paracrine biphasic control over steroidogenesis in Leydig cells (23). In the present study, no significant differences in the quantity of Leydig cells were detected between the control and treatment groups. Nonetheless, further investigation is warranted to elucidate potential variations in Leydig cell activity among the experimental groups, highlighting the necessity for additional research to clarify this aspect.

5. Conclusion

The findings from the current study suggest that implementing management techniques, such as spiking, beginning at 40 weeks of age, may effectively mitigate the decline in fertility and hatchability rates observed in broiler breeder flocks. This timely application of management strategies is crucial as it addresses the physiological and reproductive challenges associated with aging in broiler breeders, which typically result in reduced reproductive performance. By utilizing spiking methods, it is possible to enhance the overall reproductive capacity of these flocks, ultimately leading to improved results in egg fertility and hatchability. The results underscore the importance of prompt intervention in management practices to maintain reproductive efficiency, particularly as the broiler breeder population ages. In conclusion, adopting these management strategies is essential for sustaining optimal reproductive performance within broiler breeder flocks. These approaches effectively counteract the

premature decline in flock fertility while also preserving the genetic potential of breeding birds. By prioritizing reproductive efficiency, broiler breeder producers can achieve significant improvements in profitability while ensuring the sustainability of their breeding programs. Continued research into the long-term effects and best practices for these management techniques is necessary to develop comprehensive guidelines for practitioners in the field.

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Conflict of interest

The authors declare that they have no conflicts of interests. The authors also certify that there is no conflict of interest with any financial organization concerning the material discussed in the manuscript.

Animal welfare statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

As part of this experiment, all animal procedures and ethical considerations were performed following the Guide to the Care and Use of Agricultural Animals in Research and Teaching

(FASS, 2010). Also, this study was conducted according to the procedures established by the Iranian Ministry of Agriculture (Experimental Authorization No. ASRI-2016-95014).

Author contributions

Akbar Yaghobfar, Hoda Javaheri Barfourooshi, and Rezvan Yaghoubfar: Acquisition and analysis of data; **Akbar Yaghobfar and Rezvan Yaghoubfar.:** Drafting the manuscript; **Akbar Yaghobfar and Hoda Javaheri Barfourooshi.:** Critical revision of the manuscript.

Data availability statement

The data that support the findings of this study can be found, in the **javaheri, hoda (2024), "Data Rooster", Mendeley Data, V1, doi: 10.17632/wnp32zftt9.1**

References

1. Mphepya LC, van Rensburg WJ, Mpofu TJ, Mtileni BJ, Nephawe KA. Influence of male-male competition on reproductive performance and mortality of broiler breeders following intra-spiking. Poultry Science. 2019; 98(10): 4549-4554. https://doi.org/10.3382/ps/pez273
2. Volpe ALD, Slaviero A, Scher A, Molinari PV, Gerber MW, Marcon VM, Moreira F, Bianchi I, Schwegler E, Tanure CB, Peripolli V. Effect of storage time, broiler breeder strain, and age on hatchability and first-week broiler performance. Brazilian Journal of Poultry Science. 2024; 26(2): 001-010. http://dx.doi.org/10.1590/1806-9061-2024-1905
3. Sabah S, Dikmen BY. Spiking applications in broiler breeders and its effect on reproduction performance. World's Poultry Science Journal. 2023; 79 (1): 1-11. https://doi.org/10.1080/00439339.2023.2208386
4. Patil S, Khune VN, Mishra S, Singh N, Bobade MD, Bhagat V. Effect of Interspiking on Fertility and Hatchability of Vanaraja Chicken Parents. Journal of Animal Health

and Production. 2019;7(2):65–69. https://doi.org/10.17582/journal.jahp/2019/7.2.65.69.

5. Chung KM. Effect of double interspiking on fertility, behavior, and blood parameters in broiler breeder males reared under heat stress conditions. Master's Thesis, University of Tennessee submitted to University of Tennessee, Knoxville. 2010.
6. Peric L, Mitrakovic M, Tomic B, Orehvacki I, Meijerhof R. Effects of flock age, place of oviposition, and cleaning treatments of hatching eggs on hatchability in broiler breeders. Journal of Applied Poultry Research. 2022; 31 (3):100279. https://doi.org/10.1016/j.japr.2022.100279
7. Wolc A, White IMS, Olori VE, Hill WG. Inheritance of fertility in broiler chickens. Genetics Selection Evolution. 2009; 41(1):47. https://doi.org/10.1186/1297-9686-41-47
8. Haghighi M, Irani M, Jafari M, Firouzi S, Habibi H. Effect of sex ratio on the production and hatchability of broiler breeder flock. Journal of World Poultry Research. 2016; 6(1):14-17
9. Jafari M, Irani M, Haghighi M, Firouzi S, Babazadeh D. Effect of Spiking on Hatchability Levels and Testicular Weight in Broiler Breeder. Journal of World's Poultry Research. 2015; 5 (2): 29–31.
10. Sluis W. Spiking Reduces Fertility Drop in Older Flocks. Poultry World. 2014; 30 (7): 20–21.
11. Meijerhof R. Poultry Performance Plus: Intra Spiking. Accessed 13 March 2022. https://poultryperformanceplus.com/information-atabase/breeders/175-intra-spiking
12. Mangiagalli MG, Martino PA, Smajlovic T, Guidobono Cavalchini L, Marelli SP. Effect of lycopene on semen quality, fertility and native immunity of broiler breeder. British Poultry Science. 2010; 51(1): 152-157. https://doi.org/10.1080/00071660903401540
13. Parent Stock Management Manual Ross 308. Performance objectives. 2016. www.aviagen .com
14. Parent Stock Nutrition Specifications Ross 308. 2016. www.aviagen .com.
15. Castillo A, Lenzi C, Pirone A, Baglini A, Russo C, Soglia D, Schiavone A, Marzoni Fecia di Cossato M. From the Semen Collection Method to the Hatchlings: The Use of Cryopreserved Sperm from Pheasants Fed an Antioxidant-Enriched Diet. Animals 2021; 11: 2624. https://doi.org/10.3390/ani11092624

16. Fragoso JS, Pizarro Díaz M, Moreno JCA, Casanovas IP, Rodriguez-Bertos A, Barger, K. Relationships between fertility and some parameters in male broiler breeders (body and testicular weight, histology and immunohistochemistry of testes, spermatogenesis and hormonal levels). Reproduction in Domestic Animals. 2013; 48(2):345-52. https://doi.org/10.1111/j.1439-0531.2012.02161.x
17. Liang, W., He, Y., Zhu, T. et al. Dietary restriction promote sperm remodeling in aged roosters based on transcriptome analysis. BMC Genomics. 2024; 25: 680. https://doi.org/10.1186/s12864-024-10544-3
18. Laskemoen T, Fossoy F, Rudolfsen G, Lifjeld JT. Age-related variation in primary sexual characters in a passerine with male age-related fertilization success, the Bluethroat, Luscinia Svecica. Journal of Avian Biology. 2008; 39(3):322-328. https://doi.org/10.1111/j.0908-8857.2008.04178.x
19. Mfoundou JDL, Guo Y, Yan Z, Wang X. Morpho-Histology and Morphometry of Chicken Testes and Seminiferous Tubules among Yellow-Feathered Broilers of Different Ages. Veterinary Sciences. 2022; 9(9):485. https://doi.org/10.3390/vetsci9090485
20. Ordas B, Vahedi S, Seidavi A, Rahati M, Laudadio V, Tufarelli V. Effect of testosterone administration and spiking on reproductive success of broiler breeder flocks. Reproduction Domestic Animals. 2015; 50(5):820-825. https://doi.org/10.1111/rda.12595
21. Nazarpak HH, Fallah-Khair AR. Effect of spiking of young males in different percentage on economic performance of broiler breeder flock. Journal of Food, Agriculture and Environment. 2011; 9(3/4):564-569.
22. Chung KM, Smith MO, Kattesh HG. The influence of double interspiking on production and behavior in broiler breeder flocks in elevated temperature conditions. Journal of Applied Poultry Research. 2012; 21(1):63‑69. https:// doi. org/ 10.3382/ japr. 2011- 00347
23. Lengyel K, Rudra M, Berghof TVL, Leitão A, Frankl-Vilches C, Dittrich F, Duda D, Klinger R, Schleibinger S, Sid H, Trost L, Vikkula H, Schusser B, Gahr M. Unveiling the critical role of androgen receptor signaling in avian sexual development. Nature Communication. 2024;15(1):8970. https:// doi. org/ 10.1038/s41467-024-52989-w.

413 **Table 1.** Nutrition and the dietary compound of broiler chicken ration

Components of ration	Percentages
Corn	53.44
Wheat	20.85
Oil	0.08
Soybean Meal	15.93
Oysters	7.45
Di-calcium phosphate	1.26
Salt	0.31
Vitamin supplement	0.25
Mineral supplement	0.25
DL-Methionine	0.14
L-Lysine Hydrochloride	0.02
L-Threonine	0.02
Compounds calculated	
Metabolism energy (kcal / kg)	2.85
Protein (%)	14.44
Fat (%)	2.17
Linolenic acid (%)	1.40
Fiber (%)	3.84
Calcium (%)	3.30
P available (%)	0.34
Sodium (%)	0.22
Digestible arginine (%)	0.64
Digestible threonine (%)	0.46
Digestible lysine (%)	0.64
Digestible methionine (%)	0.35

414 * Vitamin and mineral supplements (in kg): Vitamin A, 4.4 g, vitamin D 3, 0.72 g, vitamin B1, 0.306 g, vitamin
415 B2, 1.5 g, vitamin B6, 0.306 g, vitamin B12, 1 g, vitamin E, 7.2 g, biotin, 1 g, vitamin K, 1 g, niacin, 2.48 g,
416 folic acid, 0.306 g, pantothenic acid, 6.08 g, choline chloride, 220 g, manganese, 2 g, iron, 10 g, zinc, 13 g,
417 iodine, 0.2 g, cobalt, 0.02 g, selenium, 0.04 g.

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Table 2. The average weights of the body and testicles between experimental groups at the end of the experiment with different management methods.

Treatment	Body weight (g)	Weight testicles (g)	The weight of the right testicle (g)	The weight of the left testicle (g)
Control (no management)	5426.7	22.90[ab]	11.50[ab]	11.47[ab]
Spiking	5940.0	34.83[a]	18.83[a]	16.33[a]
Intra-spiking	5260.0	12.10[b]	6.23[b]	5.90[b]
P-Value	0.53	0.09	0.07	0.12
SEM	419.06	6.04	3.05	3.02

[a b] Different letters indicate significant differences between experimental groups ($p \leq 0.05$).

Table 3. Average histological measurements of testicles in experimental groups at the end of the experiment period.

Treatments	Spermatogonia cell (number)	Spermatocyte cell (number)	Spermatid cell (number)	Spermatozoa cell (number)	Sertoli cell (number)	Leydig cell (number)	Seminiferous duct diameter (µm)	Germinal epithelium thickness (µm)
Control (no management)	84.07	84.00[b]	84.67[a]	84.83[a]	4.57[a]	3.30	168.47[a]	71.60[a]
Spiking	87.63	94.40[a]	93.60[a]	90.23[a]	3.33[b]	2.90	149.87[b]	52.67[b]
Intra-spiking	85.07	56.13[c]	53.57[b]	37.33[b]	3.90[ab]	3.00	114.87[c]	39.87[c]
P-Value	0.41	<0.01	<0.01	<0.01	0.005	0.32	<0.01	<0.01
SEM	1.95	3.47	4.12	5.39	0.26	0.19	4.54	1.59

[a b] Different letters indicate significant differences between experimental groups ($p \leq 0.05$).

Table 4. Main effects of management methods, spiking and intra-spiking, on the fertility of total eggs and infertility of total eggs (%) between experimental groups.

	Fertility of total eggs				**Infertility of total eggs**			
	Treatments			Means of time	Treatments			Means of time
Time	1	2	3	effect	1	2	3	effect
1	80.00	95.56	92.22	89.26[a]	20.0	4.44	7.78	10.74[c]
2	67.78	98.89	88.89	85.18[a]	32.22	1.11	11.11	14.81[c]
3	67.78	95.56	90.00	84.44[a]	32.22	4.44	10.0	15.56[cb]
4	77.78	94.44	78.89	83.70[a]	22.22	5.56	21.11	16.30[cb]
5	78.89	90.00	83.33	84.07[a]	21.11	10.0	16.67	15.93[c]
6	58.89	80.00	71.11	70.00[b]	41.11	20.00	28.89	30.00[b]
7	55.56	54.44	47.78	52.59[c]	44.45	45.56	52.22	47.41[a]
8	36.67	64.44	42.22	47.78[c]	63.33	35.56	57.77	52.22[a]
Means of Treatments	65.42	84.17	74.31	Means of Treatments	34.58	15.83	25.69	
	Treatment	Time	Treatment*Time			Treatment	Time	Treatment*Time
P-value	0.21	<0.01	0.19		P-value	0.21	<0.01	0.19
SEM	7.16	5.82	10.07		SEM	7.17	5.82	10.07

[a b] Different letters indicate significant differences between experimental groups ($p \leq 0.05$). Treatments include:1. Control; no management, 2. Spiking, 3. Intra-spiking.

Table 5. Main effects of management methods, spiking and intra-spiking, on hatchability of total eggs and Hatchability of fertile eggs (%) between experimental groups.

	Hatchability of total eggs				**Hatchability of fertile eggs**			
	Treatments			Means of time	Treatments			Means of time
Time	1	2	3	effect	1	2	3	effect
1	78.88	94.44	91.11	88.15[a]	65.81	65.93	65.74	65.83[a]
2	63.33	95.55	83.33	80.74[a]	63.31	64.34	62.64	63.43[a]
3	66.67	95.55	86.67	82.96[a]	65.81	66.67	63.99	65.49[a]
4	76.67	93.33	77.77	82.59[a]	54.70	65.87	65.74	62.10[a]
5	71.11	81.11	76.66	76.29[a]	59.07	60.29	60.43	59.93[a]
6	57.98	69.46	62.46	63.30[b]	36.16	53.50	52.29	47.32[b]
7	62.31	71.68	55.29	63.09[b]	35.57	36.15	34.07	35.26[d]
8	52.78	73.14	58.24	61.39[b]	38.92	46.77	37.41	41.03[c]
Means Treatments	66.22	84.28	73.94		52.42	57.44	55.29	
	Treatment	Time	Treatment*Time			Treatment	Time	Treatment*Time
P-value	0.18	0.002	0.64		P-value	0.14	<0.01	0.17
SEM	6.50	5.26	9.11		SEM	1.69	2.22	3.86

[a b] Different letters indicate significant differences between experimental groups ($p \leq 0.05$). Treatments include:1. Control; no management, 2. Spiking, 3. Intra-spiking.

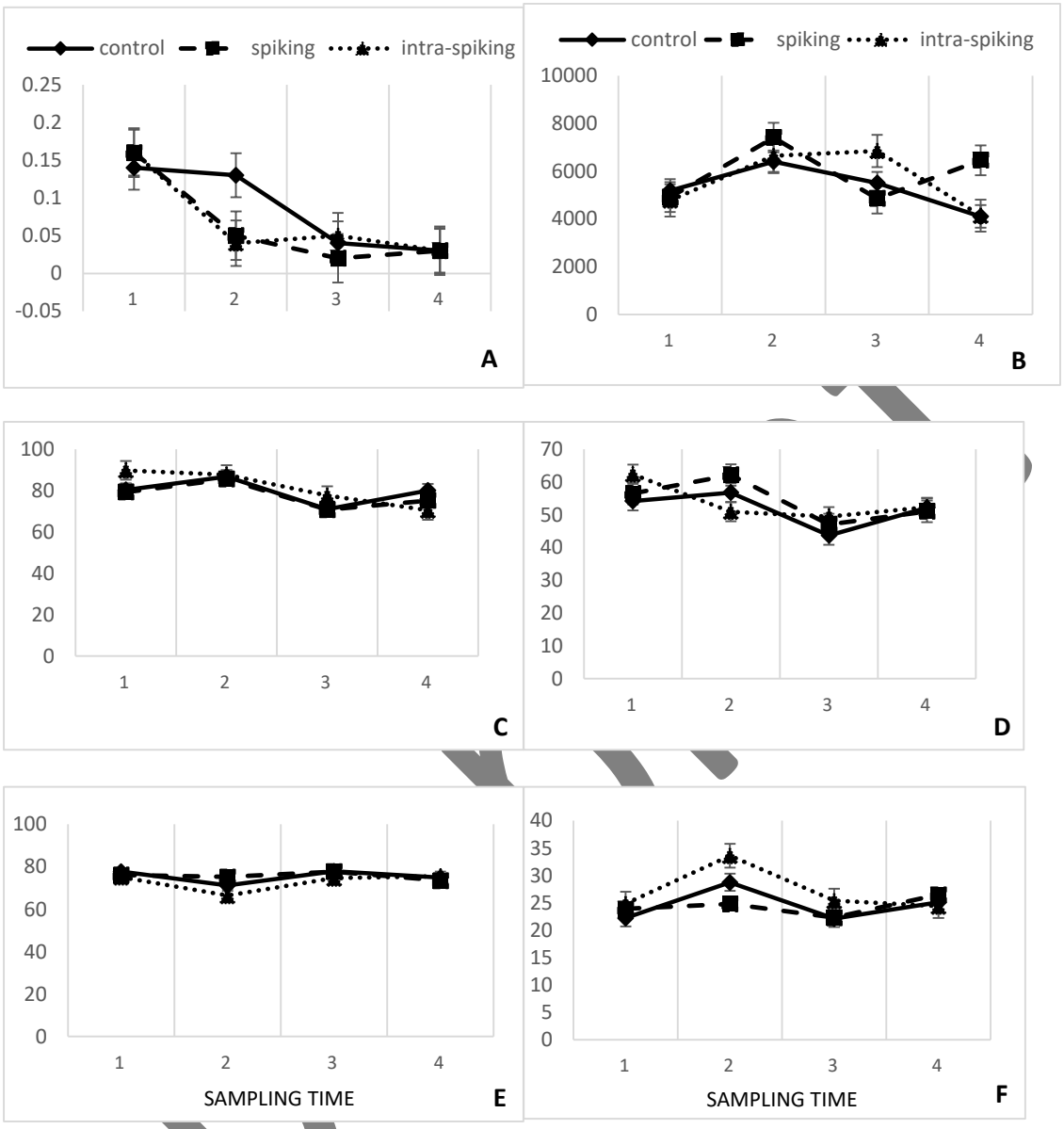
control
spiking
intra-spiking
A
B
C
D
E
F
SAMPLING TIME
SAMPLING TIME

Figure 1.

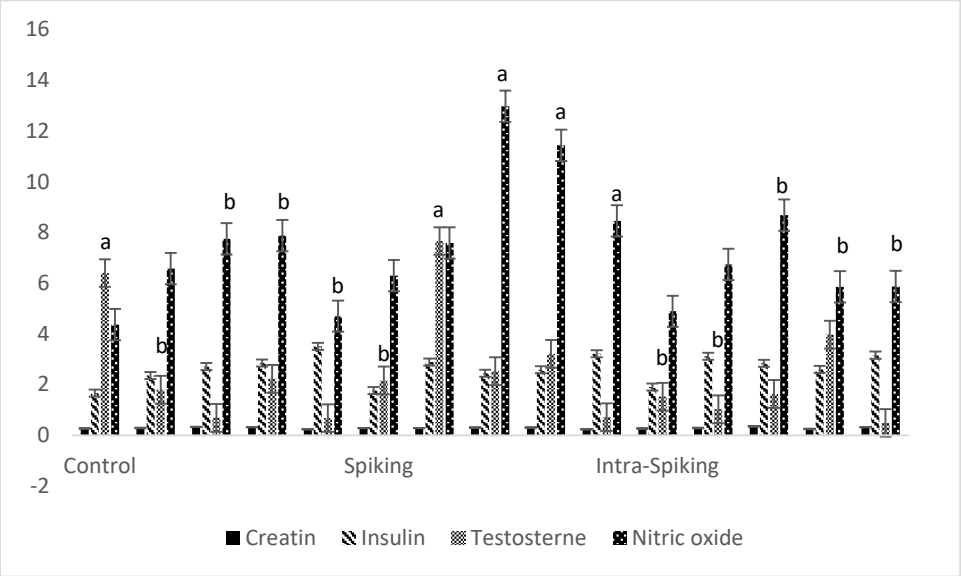

Figure 2.

Figure Captions

Figure 1. Changes in the volume (ml, **A**), concentration ($\times 10^6$, **B**), motility (%, **C**), progressive motility (%, **D**), live (%, **E**), and dead (%, **F**) of sperm between roosters in three experimental groups. Significance was considered at the level of 5% ($p \leq 0.05$).

Figure 2. Changes in the blood concentration of Creatinine (mg/dl), Insulin (µlU/ml), Testosterone (ng/ml), and Nitric oxide (µM) between cockerels in three managerial methods during the experiment. Sampling times: 40, 45, 50, 55, and 60 weeks of age. Significance was considered at the level of 5% ($p \leq 0.05$).