Feeding frequency can affect the morphology of reproductive tract in broiler breeder hens

Majid Gholami-Soltanmoradi,^{*,1} Magdalena Solka ^(a),^{1,1} Joanna Marchewka,^{1,2} Alireza Seidavi ^(a),^{*} Ross G. Cooper ^(a),[†] Mohammad Dadashbeiki,[‡] František Zigo,[§] Patryk Sztandarski ^(a),¹ Aneta Jaszczyk ^(b),¹ and Jarosław O. Horbańczuk

*Department of Animal Science, Rasht Branch, Islamic Azad University, 4147654919 Rasht, Iran; [†]Department of Physiology, Midlands State University, 9055, Gweru, Zimbabwe; [†]Department of Veterinary Science, Rasht Branch, Islamic Azad University, Rasht, Iran; [§]Department of Animal Nutrition and Husbandry, University of Veterinary Medicine and Pharmacy, 041-81 Košice, Slovakia; and [∥]Department of Animal Behavior and Welfare, Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, 05-552 Magdalenka, Poland

ABSTRACT This study examined the influence of feeding frequency on the morphology of the reproductive tract in broiler breeder hens, with a focus on the liver, ovary, and oviduct. A total of 364 hens and 52 roosters, starting at 31 wk of age and continuing until the end of their 38th week, were divided into 13 groups with varying feeding frequencies (1, 2, or 3 times daily).

Significant changes in reproductive and metabolic parameters were observed, where feeding hens twice or thrice daily resulted in significantly reduced ovary weight and follicle counts (P < 0.001), while liver weight increased (P < 0.05). These findings suggesting complex interactions between feeding practices and reproductive efficiency.

Key words: feeding frequency, ovarian follicle, ovary, liver, broiler breeder hen

INTRODUCTION

Control of body weight in broiler breeder hens during their growing and reproductive phases is crucial. Proper energy intake is essential to manage body weight and avoid the negative impacts of overfeeding, such as reduced egg production, fertility, and hatchability. Overweight hens not only suffer from lower reproductive efficiency but also face increased risks of metabolic disorders, such as fatty liver syndrome, which can further impair their productivity and health Modulating food intake at the start of laying can reduce costs and improve production efficiency (Decuypere and Kühn, 1984; Moradi et al., 2013).

Increased feeding frequency, aligned with daily feed requirements, optimizes nutrient utilization, improves enzyme production, and enhances metabolic regulation, leading to better body weight uniformity, fertility, and egg production. Regular feeding intervals can also help 2024 Poultry Science 103:104307 https://doi.org/10.1016/j.psj.2024.104307

maintain stable blood glucose levels, which is critical for sustaining reproductive processes and preventing excessive fat deposition (Taherkhani et al., 2010). However, it is important to consider the implications of restricted feeding regimens on animal welfare, particularly in relation to behavioral disorders. Restriction in feed availability can lead to heightened stress levels, expressed through abnormal behaviors such as feather pecking and increased aggression, which can compromise the welfare of the birds (Dixon et al., 2022). While such regimens in broiler breeders can achieve a laying rate comparable to well-managed commercial flocks (Van Emous et al., 2021), they must be carefully managed to mitigate potential negative impacts on bird behavior and overall welfare.

Strategic feeding schedules can prevent the overlap of metabolic heat with daily environmental heat peaks, particularly in hot climates. Feeding in the early morning is recommended to avoid increased body heat production during peak temperatures. Therefore, hens should normally be fed at 6:00 to 07:00 so that heat from nutrient metabolism is released before the daily heat peak (Moradi et al., 2013).

Based on observations it is possible to intervene with a flexible extra feeding technique, which allows to meet birds' needs in the peak production time (during egg formation) when birds' requirement fluctuates. Feeding

^{© 2024} The Authors. Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Received June 14, 2024.

Accepted September 3, 2024.

 $^{^1\!\}mathrm{M}.$ Gholami-Soltan
moradi and M. Solka share the first authorship of this article.

²Corresponding author: j.marchewka@igbzpan.pl

schedules should consider breed, strain, and environmental temperature and conditions, as these factors influence feeding time, metabolism rate and behavior (Dixon et al., 2022).

This study aimed to explore the impact of feeding frequency on the liver, ovary, and oviduct physical characteristics, morphology, and ovarian follicles development. Understanding these effects is critical not only for optimizing production efficiency but also for ensuring the long-term health and welfare of broiler breeder hens.

MATERIAL AND METHODS

The experiment was conducted over 8 wk on 364 broiler breeder hens and 52 roosters of Ross 308 strain starting at 31 wk of birds age. At this age birds average weight was $3.32 \text{ kg} \pm 0.88$. The birds had access to water *ad libitum*. A total amount of 165 g/head of feed was distributed per each day of the trial.

The groups were submitted to different dietary strategies, named from A to M. Briefly, 1 feeding strategy was assigned at random to each group of 4 pens. Under Strategy A (the control) 100% of hens' ration was given at 4:00. Under Strategy B - 50% of the ration at 4:00 and 50% at 16:00 Under Strategy C - 75% at 4:00. and 25% at 16:00. Under Strategy D - 50% at 4:00 and 50% at 12:00. Under Strategy E - 75% at 4:00 and 25% at 12:00. Under Strategy F - 75% at 6:00 and 25% at 16:00. Under Strategy G - 50% at 6:00 and 50% at 16:00. Under Strategy H - 50% at 4:00, 25% at 12:00 and 25% at 16:00. Under Strategy I - 34% at 4:00, 33% at 12:00 and 33% at 16:00. Under Strategy J - 25% at 4:00 50% at 12:00 and 25% at 16:00. Under Strategy K - 25% at 4:00, 25% at 12:00 and 50% at 16:00. Under Strategy L - 50% at 6:00, 25% at 12:00 and 25% at 16:00. Under Strategy M - 34% n at 6:00, 33% at 12:00 and 33% at 16:00.

Each pen $(3 \text{ m}^2, 1.5 \times 2 \text{ m})$ housed 8 chickens (7 hens, 1 rooster) with a drinker, feeder, and egg-laying nest, fresh, mold-free straw was used. The temperature was maintained in the range 20 to 24 °C, with 15 h of light provided daily, starting 30 minutes before feeding. Feed consumption was monitored daily, and egg production was recorded weekly per replicate.

At the trial's end at 38 wk of age, 1 hen per replicate (n = 52) was slaughtered, plucked, and necropsied. The reproductive tract, liver, and attachments were removed and dissected with a surgical blade. The whole body, liver, ovary, oviduct, and their components (magnum, isthmus, and vagina) were immediately weighed using a digital scale with 0.01 g sensitivity. Large white (LWF), small yellow (SYF), and large yellow (LYF) follicles in the left ovary were counted using a 40x loupe magnifier.

All analyses were conducted using R v2.12.2 (Foundation for Statistical Computing, 2011). Morphological data were analyzed, assuming the hen was representative of her pen. ANOVA was used to assess the impact of feeding strategy (categorical, A-M) on the examined parameters. Results for each feeding strategy were compared to Strategy A using post-hoc t-tests. Bartlett's test was applied to check the uniformity of variance for each analysis.

RESULTS AND DISCUSSION

The results of this study indicated that feeding frequency significantly influenced various metabolic and reproductive parameters in broiler breeder hens. The average daily feed intake across all groups was 148.9 g, with the control group (A) consuming the most at 162 g/d, while groups E and F consumed the least, at 134 g/d. Egg production averaged 47.1%, with the highest rate observed in group F (48.6%) and the lowest in group A (45.4%).

Liver weights were significantly higher (P < 0.01) in groups C, D, E, K, L, and M compared to groups A and B (Table 1), suggesting that increased feeding frequency contributes to greater liver mass. This finding aligns with previous research by Rosebrough (2000), which demonstrated that fasting followed by re-feeding increases liver lipogenic activity, thus potentially leading to increased liver weight. Ovary weights, on the other hand, were significantly higher (P < 0.01) in the control group (A) compared to all other groups (Table 1), indicating that reduced feeding frequency may support ovarian development.

When analyzing the reproductive tract weights, magnum weights were significantly higher (P < 0.05) in groups D and E compared to groups G and J, while isthmus weights were higher (P < 0.05) in groups E and M compared to groups A and C (Table 1). The uterus weight was notably higher (P < 0.01) in group E compared to the control group (A). These results are consistent with studies by Taherkhani et al. (2010) and Moradi et al. (2013), which found that feeding strategies can affect the reproductive morphology and metabolic functions of broiler breeders, with implications for ovulation rates and egg production.

Regarding the oviduct tract length, group E showed a significantly higher total oviduct length (P < 0.05) compared to groups A, B, C, F, G, H, J, K, and M. Additionally, the isthmus length was significantly greater (P < 0.01) in groups E, I, L, and M compared to groups A and C. Group E and L also had significantly longer uterus lengths (P < 0.05) than the control group (A) (Table 1). These findings further underscore the impact of feeding frequency on reproductive tract development.

The study also examined the effects of feeding strategies on ovarian follicles. Group B exhibited the highest number of large white follicles (**LWF**), with the control group (A) having significantly more (P < 0.01) LWF than groups C, D, E, F, G, I, J, and M. Interestingly, LWF counts in groups A, L, and M were significantly higher (P < 0.01) than in group E. Small yellow follicles (**SYF**) were significantly more numerous (P < 0.01) in the control group (A) compared to all other groups, except for groups B and H. Group B had more SYF than groups D and E, while group E had fewer SYF than group H. Large yellow follicles (**LYF**) were significantly

RESEARCH NOTE

Table 1. Effect of feeding strategy on liver, ovary, ovarian tracts weights [g] and oviduct tracts lengths [cm] in broiler breeder hens at 38 wk of age.

	Weights [g]							Lengths [cm]				
Treatment	Liver	Ovary	Total ovarian duct	Magnum	Isthmus	Uterus	Vagine	Total length	Magnum	Isthmus	Uterus	Vagine
А	67.00^{b}	76.50^{a}	60.50	28.13^{ab}	7.75^{b}	$9.00^{ m b}$	3.25	113.2^{b}	29.75	$8.90^{ m b}$	9.65^{b}	4.35
В	67.50^{b}	$69.00^{ m b}$	60.25	29.00^{ab}	8.17^{ab}	10.12^{ab}	3.37	115.6^{b}	30.25	9.17^{ab}	11.32^{ab}	4.57
С	70.53^{a}	$67.25^{\rm b}$	59.50	29.25^{ab}	7.50^{b}	10.87^{ab}	4.37	$116.1^{\rm b}$	30.62	8.70^{b}	11.90^{ab}	5.35
D	$71.00^{\rm a}$	67.50^{b}	58.75	30.50^{a}	9.12^{ab}	10.62^{ab}	4.25	117.6^{ab}	31.95	10.25^{ab}	11.60^{ab}	5.07
E	70.50^{a}	$66.00^{ m b}$	59.75	30.62^{a}	9.75^{a}	11.62^{a}	4.62	$121.3^{\rm a}$	32.05	10.97^{a}	$12.77^{\rm a}$	5.72
F	69.75^{ab}	$69.50^{ m b}$	60.25	28.25^{ab}	9.25^{ab}	10.75^{ab}	3.62	$117.2^{\rm b}$	29.67	10.32^{ab}	12.25^{ab}	4.80
G	68.75^{ab}	68.25^{b}	59.75	27.00^{b}	9.50^{ab}	10.12^{ab}	3.37	$115.3^{\rm b}$	28.77	10.77^{a}	11.32^{ab}	4.67
Н	69.25^{ab}	$69.50^{ m b}$	60.50	28.00^{ab}	$8.37^{ m ab}$	9.75^{ab}	3.37	115.7^{b}	29.52	9.52^{ab}	11.50^{ab}	4.65
Ι	68.75^{ab}	$68.50^{ m b}$	59.75	29.25^{ab}	9.25^{ab}	$9.37^{ m ab}$	3.75	117.3^{ab}	30.77	10.77^{a}	10.92^{ab}	5.05
J	68.75^{ab}	68.75^{b}	62.25	27.62^{b}	8.00^{ab}	9.12^{ab}	3.75	115.7^{b}	38.92	9.02^{ab}	$10.45^{\rm ab}$	4.82
Κ	70.25^{a}	$68.00^{ m b}$	60.25	29.05^{ab}	$8.37^{ m ab}$	9.12^{ab}	3.87	$116.1^{\rm b}$	31.15	9.45^{ab}	10.25^{ab}	5.02
L	$71.25^{\rm a}$	70.00^{b}	59.75	29.25^{ab}	9.50^{ab}	11.12^{ab}	4.12	$118.7^{\rm ab}$	30.72	$10.70^{\rm a}$	$12.40^{\rm a}$	5.15
Μ	$70.25^{\rm a}$	69.25^{b}	60.00	28.50^{ab}	9.87^{a}	10.12^{ab}	2.87	116.9^{b}	30.20	$11.07^{\rm a}$	11.37^{ab}	4.25
P-value	0.0277	< 0.0001	0.5896	0.0447	0.0188	0.0009	0.1086	0.0389	0.0765	0.0025	0.0121	0.1440
RMSE	1.74	1.70	1.66	1.47	1.07	1.04	0.77	6.54	1.54	0.95	1.09	0.66

Table 2. Effect of feeding strategy on ovarian follicles (n) in broiler breeder hens at 38 wk of age.

Treatment	Large white follicles	Small yellow follicles	Large yellow follicles	Total follicles 51.75 ^a	
А	24.75^{ab}	$15.8^{\rm a}$	$10.75^{\rm a}$		
В	25.75^{a}	13.25^{ab}	8.50^{ab}	$47.50^{\rm a}$	
С	18.75^{ecd}	$9.50^{ m bed}$	6.75^{ab}	$35.00^{ m bcd}$	
D	14.50^{ed}	$8.50^{ m cd}$	7.00^{ab}	30.00^{ed}	
E	13.50^{e}	7.00^{d}	5.25^{b}	25.75^{e}	
F	17.50^{cde}	8.75^{bcd}	8.50^{ab}	34.75^{bcd}	
G	$18.50^{ m cdf}$	$9.00^{ m bcd}$	8.25^{ab}	$35.75^{ m bcd}$	
Н	20.75^{bc}	$11.50^{ m abc}$	7.50^{ab}	$39.75^{ m bc}$	
Ι	$16.00^{ m cde}$	$9.25^{ m bcd}$	7.75^{ab}	$33.00^{ m cd}$	
J	$18.25^{\rm cde}$	$9.00^{ m bcd}$	7.00^{ab}	34.25^{bcd}	
K	$19.25^{ m bcd}$	$9.75^{ m bcd}$	$9.50^{ m ab}$	$38.50^{ m bc}$	
L	$20.25^{ m bc}$	$9.25^{ m bcd}$	$10.75^{\rm a}$	40.25^{b}	
М	$18.50^{ m cde}$	$9.25^{ m bcd}$	10.50^{a}	$38.25^{ m bc}$	
P-value	< 0.0001	< 0.0001	0.0005	< 0.0001	
RMSE	2.19	1.76	1.72	2.82	

more numerous (P < 0.01) in groups A, L, and M compared to group E. The control group (A) and group B exhibited the highest total follicle counts compared to groups D, E, and I, with group E showing the lowest total follicle count (Table 2). These results are consistent with findings by Leghari et al. (2015) and Yang et al. (2019), which suggest that a higher number of LWF, LYF, or SYF is associated with lower egg production.

The results demonstrate that the twice-daily feeding strategy produced lighter hens with fewer follicles and lighter ovaries, but with heavier liver, magnum, isthmus, uterus, and vagina weights. The increase in liver weight with more frequent feeding supports the hypothesis that feed restriction during rearing alters the metabolic characteristics and lipogenesis capacity of hens. The observed decrease in ovarian follicle numbers, particularly SYF, with increased feeding frequency aligns with the notion that feed restriction reduces the production of yellow follicles, which is critical for managing reproductive performance (Hocking et al., 1989).

Genetic selection for rapid growth in broiler breeder hens has predisposed them to metabolic and reproductive disorders, as noted by Pan et al. (2014). Overfeeding during reproductive development contributes to the formation of numerous ovarian yellow follicles, leading to the production of unsettable eggs. Additionally, the findings suggest that reproductive ability can be affected by short periods of fasting, particularly during daylight hours, as different feeding regimens influence metabolic shifts that mediate reproductive functions (Decuypere and Kühn, 1984).

Overall, this study highlighted the importance of optimizing feeding strategies to balance growth and reproductive performance in broiler breeder hens. Increased feeding frequency, especially twice or thrice-daily schedules, was shown to reduce ovary weight and follicle counts while enhancing liver and reproductive tract weights, indicating that careful management of feeding frequency could be key to improving the overall health and productivity of broiler breeder hens.

ACKNOWLEDGMENTS

We thank Dr. R. Gunton at the University of Winchester, UK, for statistical advice.

Ethics approval: The experimental protocol was approved by the Rasht Branch, Islamic Azad University, Rasht, Iran, and the experiment performed with respect to the international guidelines for research involving animals (Directive 2010/63/EU).

DISCLOSURES

The authors declares no conflicts of interest.

REFERENCES

- Decuypere, E., and E. R. Kühn. 1984. Effect of fasting and feeding time on circadian rhythms of serum thyroid hormone concentrations, glucose, liver monodeiodinase activity and rectal temperature in growing chickens. Domest. Anim. Endocrinol. 1:251–262.
- Dixon, L. M., I. C. Dunn, S. Brocklehurst, L. Baker, T. Boswell, S. D. Caughey, A. Reid, V. Sandilands, P. W. Wilson, and R. B. D'Eath. 2022. The effects of feed restriction, time of day, and time since feeding on behavioral and physiological indicators of hunger in broiler breeder hens. Poult. Sci. 101:101838.
- Hocking, P. M., D. Waddington, M. A. Walker, and A. B. Gilbert. 1989. Control of the development of the ovarian follicular hierarchy in broiler breeder pullets by food restriction during rearing. Br. Poult. Sci. 30:161–173.

- Leghari, I. H., J. Li, W. Zeng, Y. Mi, and C. Zhang. 2015. Comparative study of reproductive organs and egg quality characteristics in hyline and domestic layer breeds. Pak, Vet. J. 35:315–320.
- Moradi, S., M. Zaghari, M. Shivazad, R. Osfoori, and M. Mardi. 2013. The effect of increasing feeding frequency on performance, plasma hormones and metabolites, and hepatic lipid metabolism of broiler breeder hens. Poult. Sci. 92:1227–1237.
- Pan, Y.-E., Z.-C. Liu, C.-J. Chang, Y.-F. Huang, C.-Y. Lai, R. L. Walzem, and S.-E. Chen. 2014. Feed restriction ameliorates metabolic dysregulation and improves reproductive performance of meat-type country chickens. Anim. Reprod. Sci. 151:229–236.
- Rosebrough, R. W. 2000. Dietary protein levels and the responses of broilers to single or repeated cycles of fasting and refeeding. Nutr. Res. 20:877–886.
- Taherkhani, R., M. Zaghari, M. Shivazad, and A. Z. Shahneh. 2010. A twice-a-day feeding regimen optimizes performance in broiler breeder hens. Poult. Sci. 89:1692–1702.
- Van Emous, R. A., A. J. W. Mens, and A. Winkel. 2021. Effects of diet density and feeding frequency during the rearing period on broiler breeder performance. Br. Poult. Sci. 62:686–694.
- Yang, Y. Z., Y. Yao, Z. F. Cao, T. T. Gu, Q. Xu, and G. H. Chen. 2019. Histological characteristics of follicles and reproductive hormone secretion during ovarian follicle development in laying geese. Poult. Sci. 98:6063–6070.