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Original Article

The Effects of Breed and Body Mass Index on the Incidence of Arrested Laying Associated with Polycystic Ovarian Syndrome in Geese

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Keywords

Arrested laying, body-mass index, geese, genotype, polycystic ovarian syndrome.

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ABSTRACT

This exploratory study aimed to investigate the effects of breed and body mass index (BMI) on arrested-laying (AL) associated with polycystic ovarian syndrome (AL-PCOS) in geese. Moreover, detailed pathological assessments in geese with AL were also performed. Mast geese and Large gray geese were reared under intensive feeding conditions. Observations during the laying season showed that all AL cases (n:8) (7.84%) were in Mast geese (n:102) fed with energy and protein-dense feeds. Necropsy revealed that all Mast geese with AL also had PCOS (8/8). In Mast geese, a significant difference was observed in body weight (BW) ($p<0.0001$) and BMI ($p<0.0001$) between the AL-PCOS group and the regular laying group. Follicle classifications detected by necropsy in AL-PCOS geese showed that among the Mast geese with AL-PCOS (n=8), two had atretic and cystic follicles (25%), four had type-1 and type-2 follicles (50%), and two had necrotic type-3 follicles (25%). The research data revealed that AL-PCOS might be correlated with breed and BMI, and that overfeeding and high BMI might increase AL- PCOS in breeds with high egg production, such as Mast geese. Therefore, it is crucial for farms raising high-yielding breeds such as Mast geese to strictly follow the laying periods and feeding regimes for high profitability. No previous reports had ever investigated AL-PCOS in geese, making this study the first of its kind. Moreover, the current study is also among the few presenting data on geese reproductive physiology and pathology.

INTRODUCTION

Common reproductive disorders in avians are crucial in poultry breeding because they result in egg loss and mortality (Liu *et al.*, 2001; Rosen, 2012; Abou-Zahr, 2022). Husbandry, feeding, and management conditions generally play significant roles in the etiology of avian reproductive disorders. Reproductive disorders, such as arrested laying (AL), reproductive neoplasias, oophoritis, oviductal diseases, and ovarian cysts are common occurrences in female avians (Rosen, 2012). The disorders vary among individuals and flocks, and differences in the severity of complications causes variability in clinical symptoms. Clinical manifestations of avian reproductive diseases may include reproductive system symptoms such as AL, bloody diarrhea, and color changes in the cloaca, as well as uncertain and general clinical symptoms such as depression, lethargy, hyporexia, and fluffed feathers (Rosen, 2012).

Egg laying is a physiological process that occurs with the release of the mature follicles from the ovary in the egg production period. In this process, some specific enzymes, genes and hormones have a proteolytic role on the ovaries (Walzem & Chen, 2014; Deng *et al.,* 2022). In poultry, there are pre-hierarchical (dormant primordial and growing yolk follicles) and hierarchical follicles (preovulatory follicles) in the ovary. Before laying, the hierarchical follicles line up on the left ovary – functional during the laying period - in a particular order. The number of hierarchical follicles may vary depending on the species and genetic factors (Johnson, 2015; Yang *et al.*, 2019). Before ovulation, 4-8 large follicles of various sizes are present in the ovary, with each follicle being surrounded by a single granulosa cell layer. The number and development of follicles with different sizes is controlled by metabolic and hormonal mechanisms (Ma *et al.,* 2020).

Arrests in egg laying are associated with a multifactorial etiology and predisposing factors (Waziri *et al.,* 2019). Reportedly, the arrest in laying is a reproductive failure induced by endocrine or metabolic functional disruption. If untreated, it can lead to many severe pathological conditions, including ovarian cancer as well as reproductive system issues (Abasian *et al.,* 2018; Lee *et al.,* 2018). Previous studies found that arrest in laying was multifactorial, including factors such as genetics, nutrition type, management, endocrine and metabolic disorders, and body-mass index (Liu *et al.,* 2001; Mellouk *et al.*, 2018). Some studies detected PCOS in arrested-laying incidences in various species (Liu *et al.,* 2001; Bacon & Liu, 2003), and some previous research reported PCOS-associated AL in many poultry species, including turkeys and chickens (Liu *et al.* 2001; Lee *et al.,* 2018). However, no detailed study on this subject has been found for geese.

In our observations in previous laying seasons, more AL cases were observed in the Mast breed geese compared to Large gray breeds under the same rearing conditions. In addition, animals with AL were generally overweight and had a high BMI. Therefore, researchers suggested that the AL status could differ for chicken and goose breeds reared under control and intensive feeding conditions (Bacon & Liu, 2003; Chang *et al.* 2016; Abou-Zahr, 2022). The current study was designed to determine the effects of breed and BMI on the susceptibility to AL in two different goose breeds (Mast and Large gray) housed in the same management conditions. Moreover, detailed pathological assessments of geese with AL were performed.

MATERIALS AND METHODS

Experimental design, animal housing and farm management

This study was conducted in a commercial goose farm in Tekirdağ, Türkiye (40°57’35” N 27°33’28” E). Geese laying periods varied depending on the climatic and handling-nutrition conditions in the study area, lasting about 5.5 months between the first half of January and the end of June. The peak period for egg-laying was March.

This experimental study was approved by the Tekirdag Namik Kemal University Animal Experiment Local Ethics Committee (2023, T2023-1444). The geese included in the study had an average age of 90 weeks (88-92 weeks) and were in the second laying period. Female Mast (n:204) and Large gray (n:180) geese were raised in different sections in the same facility. A month before the laying season, the Mast and Large gray geese were divided into two groups among themselves (control and treatment-overfeeding) (Table 1). Through the laying period, the male/female ratio in both flocks was 1/3.

Table 1 – Some characteristics and AL-PCOS incidence in the geese.

Genotype	Mast						Large gray		
Groups	Control	Treatment			P_1	P_2	Control	Treatment	P_3
	non-AL-PCOS	non-AL-PCOS	AL-PCOS	Total Treatment			non-AL-PCOS	non-AL-PCOS	
Female (n)	102	94	8	102			90	90	
BW (kg)	6.36±0.06	6.73±0.08	8.13±0.12	6.84±0.08	***	***	5.48±0.06	5.56±0.06	ns
BL (cm)	82.94±0.45	83.36±0.47	84.27±1.49	83.43±0.45	ns	ns	68.14±0.41	68.31±0.38	ns
BMI (kg/cm²)	0.00093±0.00015	0.00097±0.00002	0.0011±0.00003	0.00099±0.00010	ns	**	0.001183±0.000001	0.001195±0.00001	ns
AL Suspects (%, n)	1.96 (2/102)	10.78 (11/102)			*[a]		0 (0/90)	2.22 (2/90)	ns
AL (%)	0 (0/102)	7.84% (8/102)			***[a]		0 (0/90)	0 (0/90)	ns

BW: Body weight, BL: Body length, BMI: Body mass index, AL: Arrested laying, AL-PCOS; Arrested laying associated with polycystic ovarian syndrome.

Since AL-PCOS was not observed in the control and treatment groups of Large gray geese and control groups of mast geese, the total mean value is the non-AL-PCOS mean.

P_1: Between control and treatment groups of Mast geese, P_2: Between non-AL-PCOS and AL-PCOS in the treatment group of Mast geese, P_3: Between control and treatment of Large gray geese.

***: $p<0.001$, **: $p<0.01$, *: $p<0.05$, ns: non significant. $p>0.05$.

[a] Chi-square Fisher’s exact test was used to compare the row.

The flocks were in the semi-open courtyard with a pool during the day and in the poultry house during the night. Each breed was grown in different shelters and yards. Each goose had a minimum of 1.4 m^2 shelter area and 0.7 m^2 courtyard area. The birds were exposed to natural light in the yard during the day and low lighting (30 lux between 08:00 pm and 06:00 am.) in the shelter.

All animals were fed with pellets *ad libitum* all day from one month before laying to the end of the laying period. In the treatment (overfeeding) groups, the feed contained crude protein (17.80%), crude fiber (5.60%) and crude fat (5.3%). The metabolic energy value of this feed ration was 2900 kcal/kg. In the control groups, the feed contained crude protein (15.8%), crude fiber (5.0%) and crude fat (4.0%). The metabolic energy value of this ration was 2750 kcal/kg. Mineral and vitamin values of the rations were similar in all groups.

Data collection and diagnosis of PCOS-associated AL disorder

The geese in the poultry house were monitored with an in-house camera system from the beginning of egg-laying until the end of the peak period. In the daytime, observations were made in the courtyard. Egg yield was calculated using eggs collected from nests twice a day. The animals started laying eggs approximately four weeks after the study started and egg production peaked at the end of March. Geese laying no eggs for a week and demonstrating signs such as loss of appetite, restricted movement, or refusal to mate, were potential cases of "arrested egg laying." Palpation and ultrasonographic examinations were performed on these animals to diagnose laying disorders. Ultrasonographic observations were implemented as described by Bronneberg & Taverne (2003). B-Mode transcutaneous ultrasound scanning was performed on the left side of the geese with a veterinary ultrasound device equipped with a 5-7.5 MHz linear probe (Hasvet 838, Hasvet, Türkiye). Ultrasound gel was applied and the probe was positioned on the featherless part of the abdominal skin from the left side. The presence of eggs was assessed by whether the eggshell produced a dense white echo (hyperechogenic) in the uterus, as described by Bronneberg & Taverne (2003).

Geese laying no eggs for two weeks were necropsied. The necropsies of non-laying animals provided the information on macroscopic pathological changes in the genital organs, such as the distribution pattern of the follicles on the ovaries (size and type of hierarchical distribution of the follicles), the general appearance of the follicles (turgidity, ovulation status, color, weight), and follicle types. PCOS cases were identified according to criteria defined by Bacon & Lui (2003). Follicular structures were classified according to their turgidity, color and weight, as described by Liu *et al.* (2001). Type-I follicles are yellow and lined up hierarchically according to their weight and turgidity. Type II follicles are yellow and raised. However, follicles with similar weights do not show a hierarchical order according to their weight, unlike those of Type I. Type III follicles have a whitish content and similar weight. They have no hierarchical order according to their weight. Follicles were weighed with a precision digital scale sensitive to 0.01 g.

Before necropsy, body weights and body lengths of AL geese were determined individually, and body mass index (BMI) was calculated using the following formula defined by Ferdaus *et al.* (2019):

$$\text{Body Mass Index (BMI)}: \frac{\text{Body weight (g)}}{(\text{Body lenght (cm)})^2}$$

At the end of the peak egg production period, the body weight, length and BMI of the healthy geese were also measured. Comparisons were made between the measurements of healthy and AL-PCOS geese using statistical mean ± standard deviation.

Statistical analysis

The GraphPad Prism software program (Version 8.0.2) was used for statistical analysis. Two-way ANOVA was performed to determine the main effects of the key variables (breed and nutrition type; normal or overfeeding) and their interaction (breed X nutrition type) on BW, body length (BL), and BMI. When comparing the BW, BL and BMI of geese with and without AL-PCOS, the independent t-test and the the Mann-Whitney U test were respectively used for data with a parametric and non-parametric distribution. The Chi-square Fisher exact test was used to compare the AL incidence between groups ($p<0.05$).

RESULTS

Soft or hard-shelled eggs retained in shell glands of some geese were detected by palpation of the reproductive tracts. These geese had AL-suggestive symptoms such as loss of appetite, inertia, refusal to mate and not laying eggs for one week. In other cases, egg retention, egg adhesion to the cloacal mucosa, and calcified eggs in the cloaca were determined by cloacal palpation. While 2 AL suspicious geese (1.96%) were detected in the normally-fed Mast geese, no egg retention was observed in the examination of these geese. However, in the mast geese with intensive feeding, 11 AL suspicious geese (10.78%) were detected, and 8 (7.84%) of them were confirmed to have AL when examined ($p<0.05$, 1.96% & 10.78%). Moreover, the mean BW, BL and BMI of the eight AL-PCOS Mast geese were 8.13±0.12 kg, 84.27±1.49 cm and 0.0011±0.00003, respectively. There is significant difference in BW and BMI ($p\leq0.001$), but not in BL, between AL-PCOS and non-AL-PCOS Mast geese. In Large Gray geese, no geese suspected of AL were detected in the normal feeding group, but in the intensive feeding one 2 geese (2.22%) were AL-suspicious. Upon examination, none of these 2 geese was confirmed as having AL. In normal and intensive feeding conditions, it was detected that there was no significant effect on BW, BL and BMI among Large Gray geese (Table 1). Moreover, the average levels of BW, BL and BMI in Mast and Large Gray geese in different feeding conditions are also presented in Figure 1.

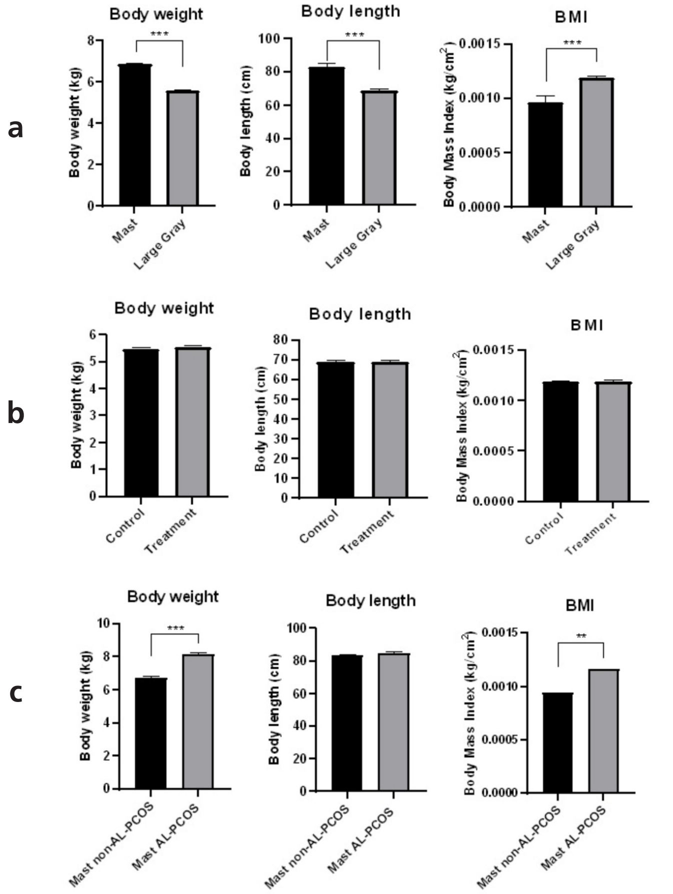

Figure 1 – Comparison of some parametric values of treatment groups of Mast and Large gray geese (a), Some parametric values in Large gray geese between control and treatment groups (b), Some parametric values in treatment group of Mast geese (c). BMI; body mass index, AL-PCOS; arrested laying associated with polycystic ovarian syndrome.

Petechiae in the oviduct mucosa, acute bleeding, and signs of infection were investigated in the necropsy of the arrested egg-laying geese. Intense fat accumulation was prominently visible in the abdomen, kidney and uterus. The necropsy operation was performed to compare the follicular status of geese, and revealed that, among AL-PCOS Mast geese (n=8), two had atretic and cystic follicles (25%) (Figure 2a), four had Type-1 and Type-2 follicles (50%) (Figure 2b), and two had Type-3 necrotic follicles (25%) (Figure 2c).

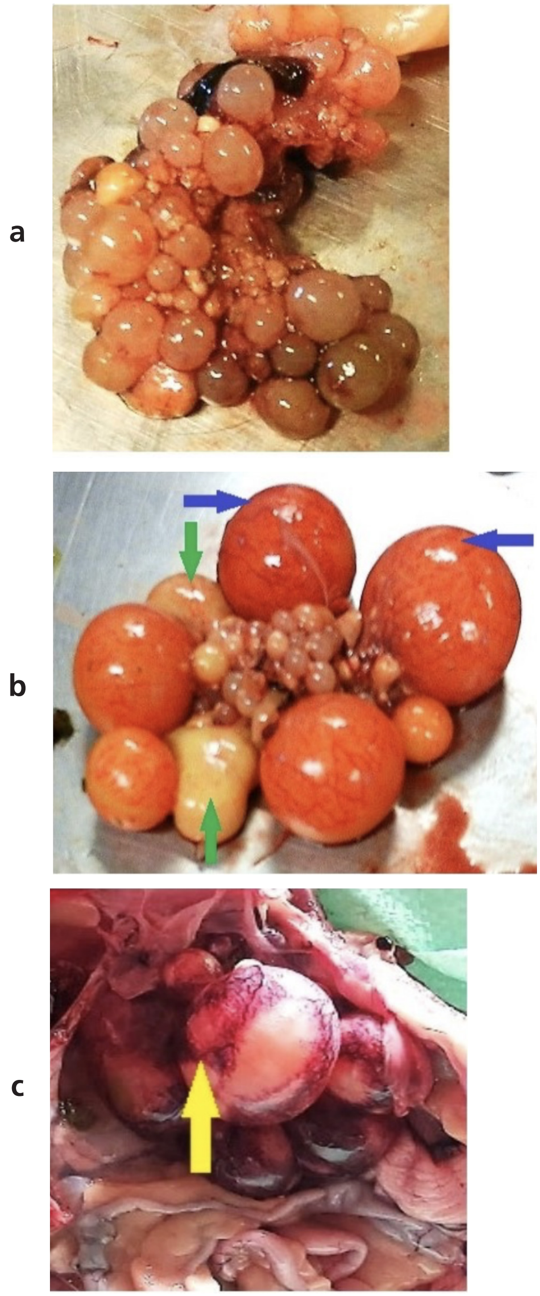

Figure 2 – The classification of follicles at different development stages on Mast geese with arrested laying associated with polycystic ovarian syndrome (AL-PCOS). Atretic and cystic follicles (a). Polyovarian follicles (Green arrow: Type 1 follicle, Blue arrow: Type II follicle) (b). Type III follicles (Yellow arrow) and their necrotic forms in the body cavity of an AL-PCOS goose. Intense fat around the kidneys, uterus and intestine tissues was evident (c).

The distrubition of follicular weight of AL-PCOS geese were determined to be 2 to 28 g in Type-1, 32 to 37 g Type-2, and 40 to 55 g in Type-3. Healthy (without AL-PCOS) Mast (n=10) and Large gray (n=10) geese in the control groups were examined for the detection of normal follicles. No pathological follicles were observed in the ovaries in the necropsy examinations of these animals. In addition, Mast geese without AL-PCOS had six hierarchical and 65-72 pre-hierarchical follicles. In the Large gray geese without AL-PCOS, the numbers of hierarchical and pre-hierarchical follicles were 5 and 53-61, respectively.

In this study, the effects of breed, nutrition type (normal or overfeeding), and breed x nutrition type interactions on BW, BL, and BMI were also evaluated. There was a significant effect of nutrition type [$p<0.0001$, F(1.380)=17.73], breed [$p<0.0001$, F(1.380)=263.8] and of the interaction [$p=0.0028$, nutrition type 0)=9.04] on BW. For BL and BMI, the breed had a significant effect [$p<0.0001$, F(1.380)=1218.0 and $p=0.0045$, F(1.380)=8.156, respectively], but there was no significancy of the nutrition type or the nutrition type × breed interaction.

DISCUSSION

Egg laying in poultry depends on certain environmental (management, feed content, henhouse conditions, etc.), hormonal, and genetic factors (Salamon, 2020; Djermanovic *et al.,* 2021). Egg formation is the outcome of integrated multi-tissue processes. Although the energy requirement for egg production in laying hens is high, hens are not very tolerant of excessive energy intake (Chen *et al.,* 2006; Walzem & Chen, 2014). Reportedly, an excessive increase of fat mass and energy intake in poultry may trigger systemic inflammation, abnormal follicular hierarchy, and several harmful metabolic chain reactions in the liver, leading to lower egg production (Walzem & Chen, 2014). Obesity due to excessive consumption in poultry is a condition that causes changes in lipid synthesis and body metabolism, affecting meat quality and potentially causing fertility problems due to imbalances in immune function and hormone production (Chen *et al.,* 2006; Walzem & Chen, 2014). Overfeeding chicken can disrupt the follicular hierarchy of the ovary, increase hyper-ovulation, block ovulation, and form necrosis in the follicles (Chen *et al.,* 2006). In the current study, all the AL-PCOS cases were diagnosed in geese fed with high energy and protein values. Furthermore, all animals with AL-PCOS had high BMI. Therefore, the study results agree with the previous study data stated above.

Ad libitum feeding or gavage practices are the most important causes of excessive weight in geese. This situation is especially noticeable in birds that show hyperphagic behavior and rapid-growing. Both breeds examined in the study were fed *ad libitum* during the laying period. The Large gray goose breed examined in the current study is of Ukrainian origin (Fisinin & Zlochevskaya, 1989). On the other hand, the Mast goose is a hybrid commercially produced in Germany in recent years to improve meat and egg yield. A growth curve comparison of the two different breeds reared under the same conditions showed that Mast geese developed earlier and had higher adult body weight than Large gray geese. This finding suggests that hybrid Mast geese may be more prone to obesity and AL-PCOS than Greater gray geese. Some studies reported that the BMI value of some animal species could provide information about body fat distribution (Iyare & Adegoke, 2008; Ferdaus *et al.,* 2019). So far, no research has been done on goose BMI. Some researchers documented that obesity could affect ovarian functions and reduce egg production in poultry (Walzem & Chen, 2014; Mellouk *et al.*, 2018). Reportedly, the BMI value and body fat ratio are significantly higher in broilers fed *ad libitum* than in those fed restrictedly (Ferdaus *et al.,* 2019).

In this study, we have calculated the effects of breed and nutrition type on BMI and their indirect correlation with AL-PCOS cases. The current study determined that the average body weight and BMI of geese with AL-PCOS were higher than the flock average. Moreover, two-way ANOVA results have indicated that there is a significant effect of breed and nutrition type on BW, but there is no significant effect of nutrition type and interaction on BL and BMI. Thus, it can be concluded that feeding conditions have greater effects in Mast geese than in Large Gray geese. In parallel, the necropsy examination showed that intense fat accumulation was particularly located in the abdominal region, around the kidneys and uterus. Therefore, the current study suggests that Mast geese are more prone to fertility problems such as obesity-related AL-PCOS. However, further studies investigating the possible relationship between AL-PCOS and BMI in geese may yield more specific results.

Reportedly, the distribution of pre-hierarchical and hierarchical follicles in the ovary of different poultry species is not similar and may vary according to species and breeds (Leghari *et al.,* 2015; Yang *et al.,* 2019). The current study found six hierarchical and 65-72 pre-hierarchical follicles in Mast geese without AL-PCOS. In the Large gray geese without AL-PCOS, hierarchical and pre-hierarchical follicle numbers were 5 and 53-61, respectively. In addition, the egg production in one laying period was higher (64 eggs/goose) in Masts than in large grays (46 eggs/goose). Previous studies documented that uninterrupted laying could cause PCOS and that progressive PCOS cases could lead to ovarian cancer (Lee *et al.,* 2018; Mellouk *et al.,* 2018). The higher egg production and hierarchical follicle number in Mast geese as compared to Large gray geese suggest that the laying intervals may have been shorter. Therefore, this situation may have triggered the PCOS reported in some cases in this study.

In the present study, the follicular orders in the ovaries morphologically determined by necropsy in Mast geese were similar to the findings of another study performed on turkeys (Liu *et al.,* 2001). In addition, geese with necrotized and atretic follicles covered with dark liquid have also been identified. Gupta *et al.* (1988) classified atresia follicles in chickens into two groups (bursting and non-bursting), based on their diameter size. Smaller, non-yolk follicles indicate non-bursting atresia. On the other hand, bursting atresia is broader than one mm in diameter and characterized by rupture of the follicular wall. In the current study, the smallest follicles were about 2 mm and the larger ones were 7 mm in diameter. Visual examination detected no egg yolk in the atretic follicles, implying there were no bursting atretic follicles. Previous studies on different species reported that the incidence of PCOS might vary depending on age (Mellouk *et al.,* 2018; Tatone *et al.,* 2021). The present study could not make such a statistical evaluation because of the similar age of the geese groups.

Previous studies on poultry breeding emphasized that physiological functions such as growth, development, egg production, and fertility depend on photoperiods and light intensity (Wenda *et al.,* 2005; Chang *et al.,* 2016; Razıq *et al.,* 2021). Bacon & Liu (2003) reported that the relationship of AL-PCOS incidence with animal ages and light intensity and duration applied to turkeys. In the present study, Mast and Large gray geese were exposed to light at 30 lux of intensity for 10 hours, in addition to daylight. The procedure was applied from one month before the laying season to the end of the laying period. The rate of AL-PCOS in this study was lower than the values reported in the different groups in the study of Bacon & Liu (2003). The difference between studies may be due to the use of different animal species.

CONCLUSIONS

According to the literature review, AL-PCOS has not been previously reported in geese. Therefore, this is the first report of PCOS observed in AL geese. Study data suggest that AL-PCOS cases may increase due to overfeeding and high BMI, especially in breeds with high egg production. Therefore, the authors suggest that it will be significant to monitor egg laying associated with feeding regimens for the profitability of hybrid and high-yielding breeds such as Mast geese. Moreover, given the scarcity of available data on the reproductive physiology and pathology of goose breeds, the current study, one of the few studies on this subject, may significantly contribute to the literature with its new findings.

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AUTHOR CONTRIBUTIONS

FTÖB: Study and manuscript preparation, conceptualization, sampling; EE: Data collection, statistical analysis, writing; HD: Sampling, data collection, statistical analysis, writing.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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