Semen Production, Testosterone Profile, and Testicular Histology of Heat Stressed Egyptian Geese Administered with L-Arginine

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ABSTRACT

Geese have a short reproductive live and ganders produce low quality semen under Egyptian heat stress condition. This study aimed to evaluate the effect of dietary L-arginine (AR) supplementation on semen production, testosterone level, and testes histological structure of Egyptian geese. Total of 30 males of Local Egyptian goose (10 months of age and 3.20±0.25 kg BW) were divided into three groups (10 in each). Ganders in the first group (control, G1) were fed ad libitum on a commercial mash feed (15.2% CP and ME of 2690 Kcal/kg) while those in G2 and G3 were fed the control diet with 0.2 and 0.4 g AR/kg, respectively. Treatment lasted for two months as a feeding period and then semen was collected for five weeks. Results showed that all semen characteristics (motility, livability, abnormality, and concentration) and total, motile, live, and normal sperm outputs/ejaculate were improved by AR-diets compared with control. The pathological score of testicular lesions were decreased by increasing AR level. Seminiferous tubule (ST) diameters (smallest, largest, and mean) were increased in both AR groups compared with control. Gander testes in G3 displayed regular arrangement of spermatogenic cell layer in ST including spermatogenic cells at gradual levels of development and large lumen of ST. Plasma testosterone level increased in G3 and G2 than in G1, respectively. Dietary supplementation of L-arginine may be used as beneficial tool for improving production and quality of semen in Egyptian male goose under heat stress conditions.

Keyword: Goose, gander, L-arginine, heat-stress, semen characteristics, testicular histology.

INTRODUCTION

The production of geese, as a domestic bird, is developing, and geese have short reproductive live; the ganders produce little ejaculate volume with low sperm livability (Łukaszewicz, 2000). Male selection is based on the ability of their sperm cells for fertilization (Donoghue, 1999 and Mellor, 2001) since bird fertility is mainly affected by semen quality, and significant differences in gander fertility were reported by several authors (Łukaszewicz, 2002; Łukaszewicz and Kraszynski, 2003).

Heat stress represent a challenge on reproductive performance and semen quality in poultry (Nawab et al., 2018) and animals (Capela et al., 2022). In heat-stressed males, increasing the ambient temperature and humidity exceed lipid peroxidation (Attia et al., 2019; El-Ratel et al., 2021). These conditions adversely affect the testicular functions causing DNA damage (Hosny et al., 2020) and a decrease in sperm output and semen quality (Türk et al., 2016). During spermatogenesis in heat-stressed animals, abnormal sperm percentage was negatively affected by impairing the mitotic division of spermatocytes (Attia et al., 2019) due to increasing free radical generation (El-Desoky et al., 2017).

In addition, quality of feeding is one of the important factors affecting the animal and poultry productivity. Under normal experimental conditions, dietary amino acids such as arginine are important to improve animal reproductive performance (Abdel–Khalek et al., 2018) and in vitro semen quality (Badr et al., 2020).

Arginine is important amino acid (2-Amino-5-guanidinopentanoic acid) in animals, and there are two types of arginine, D-arginine and L-arginine. As uricotelic species (Yuan et al., 2016), poultry cannot synthesize AR (D’Amato and Humphrey, 2010) and has insufficient arginine synthesis enzymes (Khalaji et al., 2013), thus it requires to dietary addition of AR. In laying hens, AR supplementation increased blood nitric oxide (NO) (Uyanga et al., 2022), which is important for improving reproduction (Xia et al., 2017).

In avian, AR needed for semen production (Fouad et al., 2012). Nutritionally, AR is an essential amino acid for spermatogenesis and embryonic development (Rhoads et al., 2008; Yao et al., 2008; Lassala et al., 2011) and AR administration, dietary or intravenous, has positive impacts on reproduction (Wu et al., 2009) and maternal health (Zeitoun et al., 2016). AR deficiency deranged metabolism of spermatozoa resulting in reduced motility and spermatogenesis losses (Srivastava et al., 2006). AR prevents lipid peroxidation in sperm membrane under different peroxidation conditions (Srivastava et al., 2006) and is signifying for motility, metabolism, and acrosome reaction of sperm cells (Ko and Sabanegh, 2014).

In chicken, level of testosterone and LH increased as affected by dietary AR supplementation (Sabry et al., 2016). In mice, AR had a role in testosterone anabolic action (Cremades et al., 2004) and AR supplementation improved the testicular function and semen quality in aged cocks (Abbaspour et al., 2019). Significant improvements in semen quality of Silver Montazah cock (Sabry et al., 2018).
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2016), Fayoumi and Golden Montazah cokcere (Youssef et al., 2015) were reported. Many reports cleared the role of AR in eliminating the negative impact of stress especially in hot climate (Mendes et al., 1997; Tong and Barbul, 2004).

Therefore, the current study aimed to evaluating the effect of dietary arginine supplementation on semen production, testosterone level, and histological structure of the testes in Egyptian goose ganders under heat stress conditions.

MATERIALS AND METHODS

The experimental work of this study was conducted at El-Sew Animal Experimental Station, belonging to Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agricultural during the period from May to Juley 2022.

Climatic condition:
Throughout the experimental period, average of ambient temperature (AT) and relative humidity (RH) was 37.72±3.94 °C, and 73.44±5.38, the natural photoperiod ranged between 13-14 h light and 10-11 h dark. Temperature humidity index (THI) was calculated according to LPHSI (1990): THI = db - (0.55 - 0.55 RH) (db - 58) Where: db = dry bulb temperature (° F), RH = relative humidity/100. THI values of 72 to 79 indicate mild heat stress, 79 to 89 moderate heat stress, and > 89 severe heat stress.

According to AT and RH, THI value was 93.77 during the experimental period.

Birds:
A total number of 30 local Egyptian male goose strain with 10 months of age and averaged 3.20±0.25 kg body weight. The experimental birds were divided into three groups (10 birds/group). Ganders were housed in windowless houses that contained ten pens of 2.5 x 2.5 m². Ganders of all groups were kept in an intensive system, with confinement in the house, and fed ad libitum on a commercial mash feed (15.2% CP and ME of 2690 Kcal/kg) provided reared on floor pens. Drinking water was available all day. Ingredients of the basal diet included 63% yellow corn, 15.5% soybean meal (44%), 17% wheat bran, 1.25% calcium phosphate, 1.8% calcium carbonate, 0.3% vitamin and mineral mixture, 0.3% sodium chloride, and 0.07% methionine.

Experimental design:
Three experimental groups included the control group (G1) which was fed on a basal diet without additive, while birds in G2 and G3 were fed the basal diet supplemented with 0.2 and 0.4 g L-arginine/kg (99% L-Arginine; HSN: 2922.4990, CAS No: 74-76-2, M.W. 174.20, Loba chemie pvt. Ltd., Mumbai, India) per kg, respectively. Birds were fed the experimental diets for two months as a feeding period followed by five weeks for semen collection. At the end of an experimental period of three months, five ganders were slaughtered for blood collection and histological study.

Semen collection and evaluation:
At the end of the feeding period (two months), semen was collected twice a week for five weeks as a semen collection period from 10 ganders per group. Semen was collected using the abdominal massage method after an adaptation period during the feeding interval.

On day of semen collection, ejaculate volume of each gander was measured after gel mass withdrawal (if present) using tuberculin. Sperm variables including progressive motility, livability, and abnormality were determined. Percentages of live and abnormal spermatozoa were determined according to Correa and Zavoa (1996). Phase contrast optics was used for determination of sperm variables at 40x. Sperm cell concentration was measured by weak eosin formalin (10% formalin) solution (Elkomy, 2003) using the Neubauer hemocytometer slide (Smith and Mayer, 1955).

Sperm out puts per ejaculate was calculated according to the following equations:

Total sperm output (10⁶/ejaculate) = sperm concentration (10⁶/ml) x semen volume (mL)

Motile sperm output (10⁶/ejaculate) = total sperm output x motility percentage

Live sperm output (10⁶/ejaculate) =

Normal sperm output (10⁶/ejaculate) =

Total sperm output x (abnormality percentage -100)

Testicular histology:
At the end of semen collection period, five ganders from each group were slaughtered and testes of each gander were removed for the histological study. Small specimens were taken from the median portion of each testis then fixed in 10% formalin for 14-48 h, washed by tap water for 24 h, gradually dehydrated by ethanol (50 up to 100%), cleared, routinely sectioned by microtome at 5-7 μm thickness. The sections were mounted on glass slides, deparaffinized and stained with hematoxylin and eosin to examine by a light microscope.

During the histological examination, seminiferous tubules were measured for the largest and smallest diameter by eye-piece and micrometer slide in five fields of each testis, then mean diameter was computed. Criteria for histopathologic scoring (0-3) of testis were: 0: no pathological lesion using Kruskal-Wallis and Mann-Whitney U tests; score 1: mild degenerated to sloughed germ cells, inflammation absent to minimal, absent to rare peritubular fibrosis; score 2: moderate sloughed necrotic germ cells, scattered to multifocal interstitial inflammation, moderate interstitial fibrosis; score 3: severe diffuse tubular necrosis, diffuse inflammation, diffuse interstitial fibrosis.

Testosterone profile:
Blood samples were taken from slaughtered ganders in each group (n=5) into clean test tubes with heparin. Blood samples were centrifuged at 3000 rpm for 15 min for obtaining blood plasma which was stored at -20°C until testosterone assay. Plasma testosterone concentration was determined by enzyme-immunoassay using commercial kit (Biosource-Europe S.A. 8, rue de L’Industrie. B-1400 Nivelles, Belgium). Intra- and inter-assay coefficients were 7.8 and 8.4%, respectively. The detectable limit ranged between 0.1 and 18.0 ng/ml.

Statistical analysis:
To study the effect of AR treatment, data were statistically analyzed by one-way analysis of variance (SAS, 2013). The model was as follows: Yij = μ + Ti + eij Where Yij = observation of ith bird within jth treatment, μ = overall mean, Ti = effect of jth treatment (i=1-3), eij = experimental error. All percentages were transformed to their corresponding Log10 before running the analyses. The significant differences were separated by Duncan’s Multiple Range test at P<0.05 according to Duncan (1955).

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RESULTS AND DISCUSSION

Results
Sem-en production
Physical semen characteristics
Results shown in Table 1 revealed an improvement in semen characteristics due to AR supplementation at a level of 0.2 g/kg (G2), in terms of increasing (P<0.05) semen volume, percentage of sperm progressive motility and viability, and sperm cell concentration, and decreasing (P<0.05) sperm abnormality percentage. Further improvement (P<0.05) was detected in all semen parameters studied by increasing AR level from 0.2 in G2 to 0.4 g/kg in G3.

Table 1. Effect of dietary arginine supplementation on sperm characteristics of geese during semen collection period.

<table>
<thead>
<tr>
<th>Semen characteristics</th>
<th>G1 (Control)</th>
<th>G2 (0.2 g AR)</th>
<th>G3 (0.4 g AR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen volume (ml)</td>
<td>0.56±0.03c</td>
<td>0.66±0.04b</td>
<td>0.93±0.09a</td>
<td>0.0103c</td>
</tr>
<tr>
<td>Sperm progressive motility (%)</td>
<td>61.66±1.67c</td>
<td>63.33±1.60b</td>
<td>75.00±2.89a</td>
<td>0.0090b</td>
</tr>
<tr>
<td>Sperm Livability (%)</td>
<td>71.00±2.08c</td>
<td>74.33±1.76b</td>
<td>79.66±1.20a</td>
<td>0.0319b</td>
</tr>
<tr>
<td>Sperm abnormality (%)</td>
<td>17.67±0.33c</td>
<td>17.66±0.32a</td>
<td>15.00±0.58b</td>
<td>0.0068**</td>
</tr>
<tr>
<td>Sperm cell concentration (x10^6/ml)</td>
<td>243.3±7.26c</td>
<td>295.7±12.44b</td>
<td>341.7±6.01a</td>
<td>0.0007***</td>
</tr>
</tbody>
</table>

a-c: Significant group differences at P<0.05 with different superscripts.

Total sperm outputs
Both dietary AR supplementations significantly increased number of spermatozoa, as total motile, live, and normal per ejaculate. Increasing the level of dietary AR from 0.2 to 0.4 g/kg showed significant impact on all sperm outputs per ejaculate (Table 2).

Table 2. Effect of dietary arginine supplementation on sperm outputs per ejaculate of geese during semen collection period.

<table>
<thead>
<tr>
<th>Sperm output (x10^6 ejaculate)</th>
<th>G1 (Control)</th>
<th>G2 (0.2 g AR)</th>
<th>G3 (0.4 g AR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>136.25±8.80c</td>
<td>195.16±6.82b</td>
<td>317.78±19.16a</td>
<td>0.0005***</td>
</tr>
<tr>
<td>Motile</td>
<td>84.98±6.31c</td>
<td>124.32±9.72b</td>
<td>238.50±9.96a</td>
<td>0.0009***</td>
</tr>
<tr>
<td>Live</td>
<td>96.74±7.24c</td>
<td>145.06±8.07a</td>
<td>253.14±9.87a</td>
<td>0.0004***</td>
</tr>
<tr>
<td>Normal</td>
<td>112.17±9.64c</td>
<td>160.69±9.75a</td>
<td>270.11±9.78a</td>
<td>0.0006***</td>
</tr>
</tbody>
</table>

a-c: Significant group differences at P<0.05 with different superscripts.

Pathological score of the tests
Effect of AR supplementation on restoring the testicular lesions in terms of pathological score is illustrated in figure 1. Values of pathological score were reduced (P<0.05) by both AR levels, being the lowest in G3.

Testicular histology
Effect of AR treatment on diameter of seminiferous tubules (ST) of gander testes was significant (Fig. 2). Feeding both AR-diets significantly increased the smallest diameter of ST compared with control diet. However, the largest and mean diameters of ST showed the highest values by feeding AR-diet at a level of 0.4 g/kg, followed by those fed AR-diet at a level of 0.2 g/kg, and smallest values were recorded for ganders fed the control diet. These results indicated positive impact of both AR levels on histometry of ST diameter, being wider with high than low AR level.

Testicular histogenesis
The histological examination of the testes in ganders of all groups showed diffuse, marked distorted testicular architecture with extensive sloughing of most germ epithelial cells (pyknosis and karyorrhexis) in control group (Fig. 3A). Also, marked hypo-spermatogenesis with intraluminal multinucleated giant cell formation and peritubular fibrosis admixed with few cellular infiltrates were seen in ST of the control group (Fig. 3B).

In addition, testes of ganders fed low AR level (G2) showed irregular arrangement and moderate necrosis of spermatogenic epithelial cells with peritubular and interstitial fibrosis (Fig. 4A). Feeding diet with high level of AR (G3) displayed regular arrangement of spermatogenic cell layer in ST including spermatogenic cells at gradual levels of development and large lumen of ST (Fig. 4B).
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Fig. 3. Representative photomicrograph of gander testes (seminiferous tubules, ST) showing A) pyknosis and karyorrhexis of spermatogenic cells (thin arrows), and B) intraluminal multinucleated giant cell formation (arrow) and peritubular fibrosis admixed with few cellular infiltrates (arrow) in the control group (G1). (H&E stain, 400x)

Fig. 4. Representative photomicrograph of gander testes (seminiferous tubules, ST) showing A) irregular arrangement and moderate necrosis of spermatogenic epithelial cells (thin arrow) with peritubular and interstitial fibrosis (thick arrow) in G2; B) normal architecture ST in G3. (H&E stain, 400x).

Testosterone profile
Testosterone profile in the experimental groups is illustrated in Fig. 5. Testosterone concentration in blood plasma of ganders increased (P<0.05) in G2 than in G1. Further increased (P<0.5) was observed by increasing AR to 0.4 g/kg as compared to G2 (0.2 g/kg).

Fig. 5. Effect of dietary arginine supplementation on plasma testosterone concentration of g segue at the end of experiment. (a, ..., c: Significant group differences at P<0.05 with different superscripts).

Discussion
The aim of present study was to study the effect of dietary arginine supplementation at two levels (0.2 and 0.4 g/kg on semen production, testosterone level, and histological structure of heat stressed Egyptian ganders during the interval from May to July. Results obtained from our study indicated quantitatively and qualitatively improvement in semen production of gander fed both AR diets. Semen volume, progressive motility and livability percentages, and sperm cell concentration were increased (P<0.05) while sperm abnormality percentage was decreased by both AR levels as compared to control. Also, sperm outputs per ejaculate (total, motile, live, normal) increased by both AR levels in comparison with the controls. The improvement observed in semen characteristics and sperm outputs was more remarkable in ganders fed 0.4 g/kg-diet than those fed 0.2 g/kg-diet.

When the obtained values of semen characteristics and sperm outputs per ejaculate of ganders fed the control diet under heat stress condition were compared with counterparts of Egyptian ganders under normal condition in Egypt, we found a deleterious in these values as affected by climatic condition. Semen volume, sperm motility, sperm livability percentages, a total sperm output, and motile sperm output of control gander under heat stress in our study versus those under normal conditions (El-Hanoun et al., 2017) were 0.56±0.03 vs. 0.76±0.012 ml, 61.66±1.67 vs. 69.4±1.63%, 71.00±2.08 vs. 72.5±5.8%, 137.83±8.80 vs. 179.5±6.1, and 84.98±6.31 vs. 124.5±5.8, respectively. Similar negative impacts on semen parameters was reported in roosters (Attia et al., 2019), Japanese quail (Türk et al., 2016), and rabbits (El-Desoky et al., 2017; El-Ratel et al., 2021) exposed to heat stress conditions.
In both AR treatment groups, all semen characteristics and sperm outputs were improved under heat stress condition. In agreement with the present results, dietary supplementation of AR improved sperm motility in broilers (Fouad et al., 2013; Abbaspour et al., 2019). Under in vitro conditions, AR stimulate sperm motility in different animal species (Radany et al., 1981; Patel et al., 1998) by increasing sperm metabolism (Srivastava et al., 2006). Dietary AR supplementation significantly increased semen volume, percentages of motile and live spermatozoa, and total sperm output/ ejaculate, while decreased abnormal sperm percentage in Silver Montazah cock (Sabry et al., 2016) and Fayoumi and Golden Montazah cockerels (Youssef et al., 2015) as compared to controls.

AR was reported to actively participate in spermatogenesis (Adnan, 1970; Srivastava et al., 2006). In avian, AR is required for some essential components production like Guanidinoacetic acid that improve sperm production and increase sperm motility (Fouad et al., 2012). The positive impact of AR is associated with nitric oxide (NO) (Chemineau et al., 1991) and dietary AR supplementation increased NO in blood of laying hens (Uyanga et al., 2022). NO is essential for improving the function of the reproductive system (Xia et al., 2017). Also, NO has an important role in vasodilatation (Ignarro and Napoli, 2004) leading to increasing blood flow carrying metabolites and hormones to the testes (Vonnahme et al., 2005). AR is a signify factor for sperm motility, metabolism and acrosome reaction (Ko and Sabanegh, 2014). Under in vitro conditions, AR stimulate sperm motility by increasing sperm metabolism (Srivastava et al., 2006). In sperm cells, AR improves glycolysis rate and increases ATP production rate and generation of lactate (Aydin et al., 1995). AR can increase energy production from the oxidation of fatty acid by NO (Fouad et al., 2013) or phosphorylation of creatine (Zhang et al., 2019). On the other hand, the high AR levels can increase the antioxidant capacity and decrease excessive free radical production, lipid peroxidation, and sperm cell damage (Chen et al., 2023). In this way, AR protects sperm membrane from lipid peroxidation under oxidative stress (Srivastava et al., 2006).

As such we investigate the histological structure in seminiferous tubules (ST) of gander testes. Heat stress conditions impaired the histogenesis and architecture of ST in the control group. AR treatment at a low level (0.2 g/kg) showed mild impact on elimination of the impairment of heat stress in spermatogenic layer of the ST by decreasing the pathological index in the testes. However, AR treatment at a high level (0.4 g/kg) exhibited remarkable effects on ST by improving their architectures, intact spermatogenic layer, and wide lumen with complete sperm cells as well as decreasing the pathological index in the testes. AR can promote the division of spermatogenic layer, and increase hormones secretion in poultry (Yuan et al., 2016). In chicken, AR increases LH level through GnRH neurons stimulation in hypothalamus (Basiouni, 2009; Sabry et al., 2016). Dietary AR addition stimulates the testicular function of aged cocks by increasing testis weight (Abbaspour et al., 2019). Improving the testicular function may be attributed to increasing the diameter of ST and number of Sertoli cells and Leydig cells (Ahangar et al., 2017). This finding was proved in our study in terms of increasing the smallest, largest, and mean diameters of ST by dietary AR administration.

Finally, AR treatment in the current study significantly increased level of blood testosterone. In chicken, level of testosterone and LH increased as affected by dietary AR supplementation (Sabry et al., 2016) and AR had role in testosterone anabolic action in mice (Cremades et al., 2004). Dietary AR addition stimulates the testicular function of aged cocks by increasing testosterone production (Abbaspour et al., 2019). Several reports stated a relationship between testosterone level and quality of semen (Zeman et al., 1986; Cecil and Bakst, 1988). In avian male, testis and reproductive tract were development under the control of estrogen and testosterone (Rivas et al., 2002; Akingbemi, 2005).

CONCLUSION
According to the obtained results, dietary L-arginine (0.2 and 0.4g/kg) supplementation promotes beneficial effects by elimination of the negative effects of heat stress effects on testicular function to produce good quality semen in Egyptian male geese. These impacts based on the direct effect of arginine in a direct pathway by increasing testosterone secretion, spermatogenesis, and semen quality, or in an indirect pathway by improving antioxidant status, immunity, and body confirmation of male geese under heat stress conditions in Egypt.

REFERENCES


