

Journal of Animal and Poultry Production

Journal homepage & Available online at: www.jappmu.journals.ekb.eg

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

Madian, H. A.; M. A. E. Hassan; Eman S. El-Hadad*; M. F. Saad; A. M. El-Shhat and Entesar Z. Eliraqy

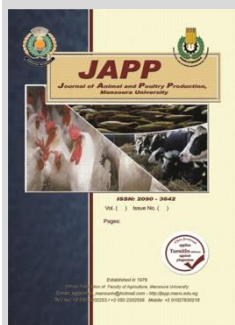


Animal Production Research Institute, Agricultural Research Center, Dokki, Giza 12618, Egypt.

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 geese (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 geese under heat stress conditions.

Keyword: Geese, 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 (Lukaszewicz, 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 (Lukaszewicz, 2002; Lukaszewicz and Kruszynski, 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 (Khajali *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 supplementations 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.*,

* Corresponding author.

E-mail address: dremanhadad@hotmail.com

DOI: 10.21608/jappmu.2023.238114.1094

2016), Fayoumi and Golden Montazah cockere (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 geese ganders under heat stress conditions.

MATERIALS AND METHODS

The experimental work of this study was conducted at El-Serw Animal Experimental Station, belonging to Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agricultural during the period from May to July 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 geese 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% di-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: 74-79-3: C₆H₁₄N₄O₂, 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 40×. 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) =

total sperm output x livability percentage

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% neutral 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 hematoxyline 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 micrometric 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: $Y_{ij} = \mu + T_i + e_{ij}$ Where Y_{ij} = observation of i^{th} bird within j^{th} treatment, μ = overall mean, T_i = effect of i^{th} treatment (i:1-3), e_{ij} = 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).

RESULTS AND DISCUSSION

Results

Semen 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 livability, 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.

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

^{a, b, 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 of 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.

Sperm output (x10 ⁶ /ejaculate)	G1 (Control)	G2 (0.2 g AR)	G3 (0.4 g AR)	P-value
Total	136.25±8.80 ^c	195.16±6.82 ^b	317.78±9.16 ^a	0.0005***
Motile	84.98±6.31 ^c	124.32±9.72 ^b	238.50±9.96 ^a	0.0009***
Live	96.74±7.24 ^c	145.06±8.07 ^b	253.14±9.87 ^a	0.0004***
Normal	112.17±9.64 ^c	160.69±9.75 ^b	270.11±9.78 ^a	0.0006***

^{a, b, c}: Significant group differences at $P<0.05$ with different superscripts.

Pathological score of the testes

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.

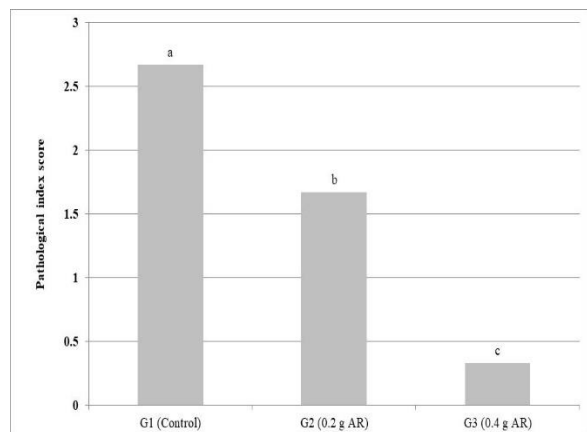


Fig. 1. Effect of dietary arginine supplementation on pathological score in testes of geese at the end of experiment. (Different letter indicates significant differences at $P<0.05$).

Testicular histometry

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.

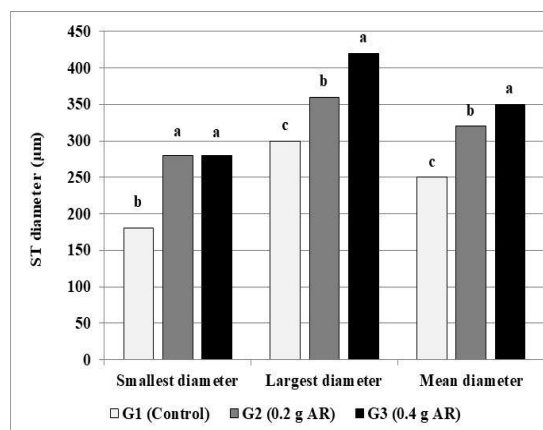


Fig. 2. Effect of dietary arginine supplementation on diameter of seminiferous tubules in the testes of geese at the end of the experiment. (Different letter indicate significant differences at $P<0.05$)

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).

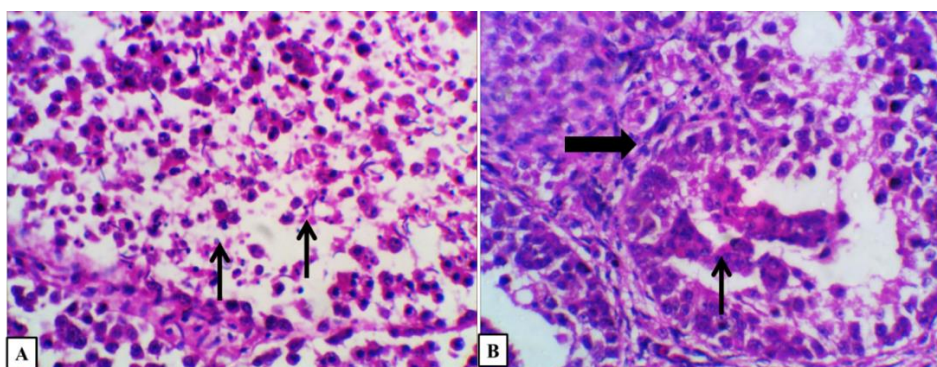


Fig. 3. Representative photomicrograph of gander testes (seminiferous tubules, ST) showing A) pyknosis and karyorrhexis of spermatogenic cells (thin arrows), and B) intraluminal multinucleated giant cell formation (arrow) and peritubular fibrosis admixed with few cellular infiltrates (arrow) in the control group (G1). (H&E stain, 400x)

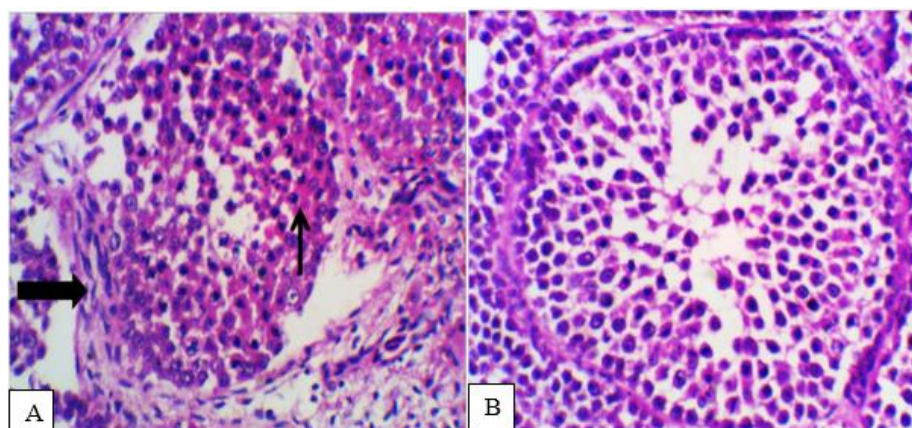


Fig. 4. Representative photomicrograph of gander testes (seminiferous tubules, ST) showing A) irregular arrangement and moderate necrosis of spermatogenic epithelial cells (thin arrow) with peritubular and interstitial fibrosis (thick arrow) in G2; B) normal architecture ST in G3. (H&E stain, 400x).

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).

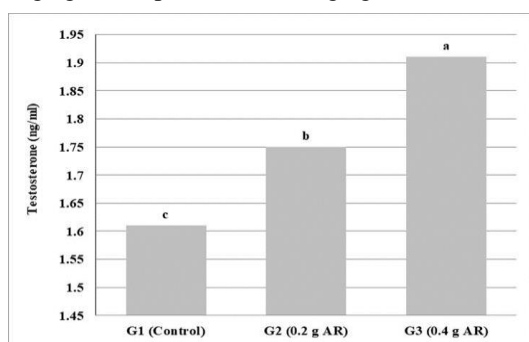


Fig. 5. Effect of dietary arginine supplementation on plasma testosterone concentration of geese at the end of experiment. (a, c: Significant group differences at $P<0.05$ with different superscripts).

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. $69.4\pm 1.63\%$, 71.00 ± 2.08 vs. $72.5\pm 5.8\%$, 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

- Abbaspour B., Sharifi S.D., Ghazanfari S., Mohammadi-Sangcheshmeh A., Honarbakhsh S. (2019). The effect of L-arginine and flaxseed on plasma testosterone concentration, semen quality and some testicular histology parameters in old broiler breeder roosters. *Theriogenology*, 128: 101-109. <https://doi.org/10.1016/j.theriogenology.2019.01.034>.
- Abdel-Khalek A.E., Khalil W.A., Ashmawy T.M., Abd El-Rafaa, Liza A. (2018). Performance and Puberty of Ram Lambs Produced From Ewes Treated with Arginine. *J. Anim. and Poultry Prod., Mansoura Univ.*, 9(3): 183-190. <https://doi.org/10.21608/JAPPMU.2018.39793>.
- Adnan M. (1970). Effect of arginine on oligospermia. *Fertil and Steril*, 21:217-219. [https://doi.org/10.1016/S0015-0282\(16\)37384-8](https://doi.org/10.1016/S0015-0282(16)37384-8).
- Ahangar M., Asadzadeh S., Rezaei-pour V., Zareh Shahneh A. (2017). Effects of L-Arginine supplementation on semen quality, testosterone concentration and testes histological parameters of Ross 308 breeder roosters. *Asian Pacific Journal of Reproduction*, 6(3): 133-135. <https://doi.org/10.12980/apjr.6.20170307>.
- Akingbemi B.T. (2005). Estrogen regulation of testicular function. *Reprod. Biol. Endocrinol.*, 3: 51. <https://doi.org/10.1186/1477-7827-3-51>.
- Attia Y.A., El-Naggar A.S., Abou-Shehema B.M., Abdella, A.A. (2019). Effect of supplementation with trimethylglycine (betaine) and/or vitamins on semen quality, fertility, antioxidant status, DNA repair and welfare of roosters exposed to chronic heat stress. *Animals*, 9: 547. <https://doi.org/10.3390/ani9080547>.
- Aydin S., Inci O., Alagöl, B. (1995). The role of arginine, indomethacin and kallikrein in the treatment of oligoasthenospermia. *Int. Urol. Nephrol.*, 27(2):199-202. <https://doi.org/10.1007/BF02551320>.
- Badr M.R., Abdel-Khalek A.E., Sakr A.M., Hegazy M.M., Rawash, Z.M. (2020). Effect of level and time of L-arginine addition to semen extender on the freezability and fertilizing potentials of buffalo spermatozoa. *Assiut Vet. Med. J.*, 66(166): 19-30. <https://doi.org/10.21608/avmj.2020.167299>.

- Basiouni G.F. (2009). The effect of feeding an extra amount of arginine to local Saudi hens on luteinizing hormone secretion. *J. Biol. Sci.*, 9: 617-620. <https://doi.org/10.3923/jbs.2009.617.620>.
- Capela L., Leites I., Romão R., Lopes-da-Costa L. and Pereira R.M.L.N. (2022). Impact of Heat Stress on Bovine Sperm Quality and Competence. *Animals (Basel)*, 12(8): 975. <https://doi.org/10.3390/ani12080975>.
- Cecil H.C., Bakst M.R. (1988). Testosterone concentrations in blood and seminal plasma of turkeys classified as high or low semen producers. *Poult. Sci.*, 67(10): 1461-1464. <https://doi.org/10.3382/ps.0671461>.
- Chemineau D.Y., Cogine Y., Guerin P., Valtet J.C. (1991). Training manual on Artificial insemination in sheep and goat. *FAO. Anim. Prod. and Heal.*, 3: 83-90. <https://cgspage.cgiar.org/bitstream>.
- Chen Y., Zhang B., Wang B., Zhang M., Fan W., Li W. (2023). Effects of dietary arginine supplementation on production performance, serum biochemicals, antioxidant capacity, and immunity of laying Wulong geese. *Poult. Sci.*, 102(7): 102727. <https://doi.org/10.1016/j.psj.2023.102727>.
- Correa J.R., Zavos P.M. (1996). Preparation and recovery of frozen-thawed bovine spermatozoa via various sperm selection techniques employed in assisted reproductive technologies. *Theriogenology.*, 46(7): 1225-1232. [https://doi.org/10.1016/s0093-691x\(96\)00293-2](https://doi.org/10.1016/s0093-691x(96)00293-2).
- Cremades A., Ruzafa C., Monserrat F., López-Contreras A.J., Peñafiel, R. (2004). Influence of dietary arginine on the anabolic effects of androgens. *J. of Endocr.*, 183: 343-351. <https://doi.org/10.1677/joe.1.05783>.
- D'Amato J.L., Humphrey B.D. (2010). Dietary arginine levels alter markers of arginine utilization in peripheral blood mononuclear cells and thymocytes in young broiler chicks. *Poult. Sci.*, 89(5): 938-947. <https://doi.org/10.3382/ps.2009-00611>.
- Donoghue A.M. (1999). Prospective approaches to avoid flock fertility problems: predictive assessment of sperm function traits in poultry. *Poult. Sci.*, 78(3): 437-443. <https://doi.org/10.1093/ps/78.3.437>.
- SAS (2013). *Statistical Analysis Software. Users' Guide Statistics Version 9.4.* SAS Institute Inc., Cary.
- Duncan I.B. (1955). Gas gangrene. *Med Illus.*, 9(3): 163-168. <https://doi.org/10.1111/j.1742-1241.1955.tb02117.x>.
- El-Desoky N.I., Hashem N.M., Elkomy A., Abo-elezz Z.R. (2017). Physiological response and semen quality of rabbit bucks supplemented with Moringa leaves ethanolic extract during summer season. *Animal*, 11: 1549-1557. <https://doi.org/10.1017/S1751731117000088>.
- El-Hanoun A.M., Fares Wesam A., Attia Y.A., Abdella M.M. (2017). Effect of magnetized well water on blood components, immune indices and semen quality of Egyptian male geese. *Egypt. Poult. Sci.*, 37(I): 91-103. <https://doi.org/10.21608/EPSJ.2017.6031>.
- Elkomy A.E. (2003). Physiological studies on Gibberellic Acid (GA) and reproductive functions 3 of adult fowl. Ph.D. Thesis, Faculty of Agriculture, Alexandria University.
- El-Ratel, I.T., Attia, K.A.H., El-Raghi, A.A. and Fouda, S.F. (2021). Relief of the negative effects of heat stress on semen quality, reproductive efficiency and oxidative capacity of rabbit bucks using different natural antioxidants. *Anim. Biosci.*, 34(5): 844-854. <https://doi.org/10.5713/ajas.20.0258>.
- Fouad A.M., El-Senousey H.K., Yang X.J., Yao J.H. (2012). Role of dietary L-arginine in poultry production. *Int. J. Poult. Sci.*, 11: 718-729. <https://doi.org/10.3923/ijps.2012.718.729>.
- Fouad A.M., El-Senousey H.K., Yang X.J., Yao J.H. (2013). Dietary L-arginine supplementation reduces abdominal fat content by modulating lipid metabolism in broiler chickens. *Animal*, 7: 1239-1245. <https://doi.org/10.1017/S1751731113000347>.
- Hosny N.S., Hashem N.M., Morsy A.S., Abo-elezz Z.R. (2020). Effects of organic selenium on the physiological response, blood metabolites, redox status, semen quality, and fertility of rabbit bucks kept under natural heat stress conditions. *Front. Vet. Sci.*, 7: 290. <https://doi.org/10.3389/fvets.2020.00290>.
- Ignarro L.J., Napoli C. (2004). Novel features of nitric oxide, endothelial nitric oxide synthase, and atherosclerosis. *Curr. Atheroscler. Rep.*, 6(4): 281-287. <https://doi.org/10.1007/s11883-004-0059-9>.
- Khajali F., Basoo H., Faraji M. (2013). Estimation of Arginine Requirements for Male Broilers Grown at High Altitude from One to Twenty-one Days of Age. *J. of Agric. Sci. and Tech.*, 15: 911-917. <https://api.semanticscholar.org/CorpusID:44238101>.
- Ko E.Y., Sabanegh E.S. (2014). The role of nutraceuticals in male fertility. *Urologic Clinics of North America*, 41: 181-193. <https://doi.org/10.1016/j.ucl.2013.08.003>.
- Lassala A., Bazer F.W., Cudd T.A., Datta S., Keisler D.H., Satterfield M.C., Spencer T.E., Wu G. (2011). Parenteral Administration of L-Arginine Enhances Fetal Survival and Growth in Sheep Carrying Multiple Fetuses. *J. Nutr.*, 141: 849-855. <http://dx.doi.org/10.3945/jn.111.138172>.
- LPHSI (1990). *Livestock and poultry heat stress indices agriculture engineering technology guide.* Clemson University, Clemson, SC, USA
- Lukaszewicz E. (2000). The effect of semen filtration on morphology and fertilising ability of gander spermatozoa. *British Poultry Science*, 41: suppl.(17). <https://doi.org/10.1080/00071660050148543>.
- Lukaszewicz E. (2002). An effective method for freezing White Italian gander semen. *Theriogenology*, 58(1): 19-27. [https://doi.org/10.1016/s0093-691x\(01\)00690-2](https://doi.org/10.1016/s0093-691x(01)00690-2).
- Lukaszewicz E., Kruszyński W. (2003). Evaluation of fresh and frozen-thawed semen of individual ganders by assessment of spermatozoa motility and morphology. *Theriogenology*, 59(7): 1627-1640. [https://doi.org/10.1016/s0093-691x\(02\)01209-8](https://doi.org/10.1016/s0093-691x(02)01209-8).
- Mellor S. (2001). Selecting males by sperm quality. *World Poultry*, 17: 32-34.
- Mendes A.A., Watkins S.E., England J.A., Saleh E.A., Waldroup A.L., Waldroup P.W. (1997). Influence of dietary lysine levels and arginine:lysine ratios on performance of broilers exposed to heat or cold stress during the period of three to six weeks of age. *Poultry Science*, 76: 472-481. <https://doi.org/10.1093/ps/76.3.472>.
- Nawab A., Ibtisham F., Li G., Kieser B., Wu J., Liu W., Zhao Y., Nawab Y., Li K., Xiao M., An L. (2018). Heat stress in poultry production: Mitigation strategies to overcome the future challenges facing the global poultry industry. *J. Therm. Biol.*, 78: 131-139. <https://doi.org/10.1016/j.jtherbio.2018.08.010>.
- Patel A.B., Srivastava S., Phadke R.S., Govil G. (1998). Arginine activates glycolysis of goat epididymal spermatozoa: An NMR study. *Biophys J.*, 75(3): 1522-1528. [https://doi.org/10.1016/S0006-3495\(98\)74071-8](https://doi.org/10.1016/S0006-3495(98)74071-8).

- Radany E.W., Atherton R.W., Forrester I.T. (1981). Arginine uptake by rabbit spermatozoa. Arch. Biochem. Biophys., 210: 770-774. [https://doi.org/10.1016/0003-9861\(81\)90244-7](https://doi.org/10.1016/0003-9861(81)90244-7).
- Rhoads J.M., Liu Y., Niu X., Surendran S., Wu G. (2008). Arginine Stimulates cdx2-Transformed Intestinal Epithelial Cell Migration via a Mechanism Requiring Both Nitric Oxide and Phosphorylation of p70 S6 Kinase. J. Nutr., 138(9): 1652-1657. <https://doi.org/10.1093/jn/138.9.1652>.
- Rivas A., Fisher J.S., McKinnell C., Atanassova N., Sharpe R.M. (2002) Induction of Reproductive Tract Developmental Abnormalities in the Male Rat by Lowering Androgen Production or Action in Combination with a Low Dose of Diethylstilbestrol: Evidence for Importance of the Androgen-Estrogen Balance, Endocrinology, 143(12): 4797-4808. <https://doi.org/10.1210/en.2002-220531>.
- Sabry M.M., EL Salmony A.E., Soliman M.M., EL Zyat A.A., Hannan S.M. (2016). Effect of dietary arginine supplementation on some hormones and its relation to performance of silver montazah chicken. 2- The effect on laying duration. Egyptian Poultry Science Journal, 36(1): 263-278. <https://doi.org/10.21608/epsj.2016.33263>.
- Smith J.T., Mayer D.T. (1955). Evaluation of sperm concentration by the hemacytometer method; comparison of four counting fluids. Fertil Steril., 6(3): 271-275. [https://doi.org/10.1016/s0015-0282\(16\)31987-2](https://doi.org/10.1016/s0015-0282(16)31987-2).
- Srivastava S., Desai P., Coutinho E., Govil G. (2006). Mechanism of Action of L-arginine on the Vitality of Spermatozoa is Primarily Through Increased Biosynthesis of Nitric Oxide. Biology of Reproduction, 74(5): 954-958. <https://doi.org/10.1095/biolreprod.105.046896>.
- Tong B.C., Barbul A. (2004). Cellular and physiological effects of arginine. Mini Rev Med Chem., 4(8): 823-832. <https://doi.org/10.2174/1389557043403305>.
- Türk G., Çeribaşı A.O., Şimşek Ü.G., Çeribaşı S., Güvenç M., Özer Kaya Ş., Çiftçi M., Sönmez M., Yüce A., Bayraktar A., Yaman M., Tonbak F. (2016). Dietary rosemary oil alleviates heat stress-induced structural and functional damage through lipid peroxidation in the testes of growing Japanese quail. Anim. Reprod. Sci., 164: 133-143. <https://doi.org/10.1016/j.anireprosci.2015.11.021>.
- Uyanga V.A., Xin Q., Sun M., Zhao J., Wang X., Jiao H., Onagbesan O.M., Lin H. (2022). Research Note: Effects of dietary L-arginine on the production performance and gene expression of reproductive hormones in laying hens fed low crude protein diets. Poultry Sci., 101(5): 101816. <https://doi.org/10.1016/j.psj.2022.101816>.
- Vonnahme K.A., Wilson M.E., Li Y., Rupnow H.L., Phernetton T.M., Ford S.P., Magness R.R. (2005). Circulating levels of nitric oxide and vascular endothelial growth factor throughout ovine pregnancy. J. Physiol., 565(Pt1): 101-109. <https://doi.org/10.1113/jphysiol.2004.082321>.
- Wu G., Bazer F.W., Davis T.A., Kim S.W., Li P., Marc Rhoads J., Carey Satterfield M., Smith S.B., Spencer T.E., Yin Y. (2009). Arginine metabolism and nutrition in growth, health and disease. Amino Acids, 37(1): 153-168. <https://doi.org/10.1007/s00726-008-0210-y>.
- Xia W., Fouad A.M., Chen W., Ruan D., Wang S., Fan Q., Wang Y., Cui Y., Zheng C. (2017). Estimation of dietary arginine requirements for Longyan laying ducks. Poultry Sci., 96: 144-150. <https://doi.org/10.3382/ps/pew205>.
- Yao K., Yin Y.L., Chu W., Liu Z., Deng D., Li T., Huang R., Zhang J., Tan B., Wang W., Wu G. (2008). Dietary Arginine Supplementation Increases mTOR Signaling Activity in Skeletal Muscle of Neonatal Pigs. Journal of Nutrition, 138(5): 867. <https://doi.org/10.1093/jn/138.5.867>.
- Youssef S.F., Shaban S.A.M., Inas I. Ismail (2015). Effect of L-arginine supplementation on productive, reproductive performance, immune response and gene expression in tow local chicken strains: 1- Egg production, reproductive performance and immune response. Egypt. Poultry Sci., 35: 573-590. ISSN: 2090-0570 (On line).
- Yuan C., Bu X.C., Yan H.X., Lu J.J., Zou X.T. (2016). Dietary L-arginine levels affect the liver protein turnover and alter the expression of genes related to protein synthesis and proteolysis of laying hens. Poultry Sci., 95(2): 261-267. <https://doi.org/10.3382/ps/pev314>.
- Zeitoun M., Al-Ghoneim A., Al-Sobayil K., Al-Dobaib S. (2016). L-Arginine Modulates Maternal Hormonal Profiles and Neonatal Traits during Two Stages of Pregnancy in Sheep. Open Journal of Animal Sciences, 6: 95-104. <http://dx.doi.org/10.4236/ojas.2016.62012>.
- Zeman M., Kosutzký J., Bobáková E. (1986). Testosterone concentration in the seminal plasma of cocks. Br. Poultry Sci., 27(2): 261-266. <https://doi.org/10.1080/00071668608416879>.
- Zhang L., Li J.L., Wang X.F., Zhu X.D., Gao F., Zhou G.H. (2019). Attenuating effects of guanidinoacetic acid on preslaughter transport-induced muscle energy expenditure and rapid glycolysis of broilers. Poultry Sci., 98: 3223-3232. <https://doi.org/10.3382/ps/pez052>.

إنتاج السائل المنوي، صورة التستوستيرون والتركيبة النسيجية للخصية في الأوز المصري المجهد حرارياً والمعالج بـ إL-أرجينين

هشام أحمد مدين ، محمود عبدالفتاح السيد حسن ، إيمان سعيد الحداد* ، محمد فهيمي سعد ، عبدالغني محمد الشحات و إنتصار زكريا العراقي

معهد بحوث الإنتاج الحيواني ، مركز البحوث الزراعية ، جيزة ١٢٦١٨ ، مصر.

المخلص

يتمتع الأوز بحياة تناسلية قصيرة وينتج ذكر الأوز سائل منوي منخفض الجودة تحت ظروف الإجهاد الحراري المصرية. تهدف الدراسة إلى تقييم تأثير إضافة الأرجينين على إنتاج السائل المنوي ومستوى هرمون التستوستيرون والتركيبة النسيجية للخصية الأوز المصري. تم تقسيم ٣٠ ذكر من الأوز المصري المحلي (عمر ١٠ أشهر و ٢٠،٣ ± ٢٥،٠ كجم وزن الجسم) إلى ثلاث مجموعات (١٠ في كل مجموعة). تمت تغذية ذكور الأوز في المجموعة الأولى (مجموعة الكنترول) بالتغذية الحرة على مخلوط علف تجاري (١٥،٢٪ بروتين خام و ٢٦٩٠ كيلو كالوري/كجم طاقة ممثلة) في حين تم تغذية المجموعة الثانية والثالثة على علفية مجموعة الكنترول مع إضافة ٠،٢ و ٠،٤ جرام أرجينين/كجم ، على التوالي. استمرت المعاملة لمدة شهرين كفترة تغذية ثم تم جمع السائل المنوي لمدة خمسة أسابيع. أظهرت النتائج أن جميع خصائص السائل المنوي (القدرة على الحركة ، الحيائية ، الشواذ والتركيبة/مل) والمنتج من الحيوانات المنوية الكلية والمتحركة والحيية والطبيعية في كل قففة قد تحسنت بالعلائق المحتوية على أرجينين مقارنة بالكنترول. انخفض pathological score للخصية مع زيادة مستوى إضافة الأرجينين. زادت أقطار الأنبيبات المنوية (الأصغر ، الأكبر والمتوسط) في خصيتي ذكور الأوز في كلا مجموعتي الأرجينين مقارنة بالكنترول. أظهرت خصيتي ذكور الأوز غاندر في المجموعة الثالثة ترتيباً منتظماً لطبقة الخلايا المنوية في الأنبيبات المنوية محتوية خلايا منوية عند مستويات تدريجية من التطور وتجويف كبير للأنبيبات المنوية. ارتفع هرمون التستوستيرون في بلازما دم ذكور الأوز في (P<0.05) المجموعة الثالثة والثانية عن الأولى. يمكن استخدام إضافة الأرجينين كأداة مفيدة في تحسين إنتاج السائل المنوي من ذكور الأوز تحت ظروف الإجهاد الحراري المصرية.

الكلمات الدالة: ذكور الأوز ، إL-أرجينين ، الإجهاد الحراري ، صفات السائل المنوي ، هستولوجيا الخصية