Research Article

Impact of Dietary Arginine Supplementation on Hemato-Biochmical, Histological, Antioxidant, and Immunity Variables of Geese under Heat Stress Condition in Egypt

Eman Said El-Hadad*, Hesham Ahmed Madian, Mahmoud A.E. Hassan, Mohamed Fahmy Saad, Abdelghany M. El-Shhat, Entesar Zakaria Eliraqy

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

Abstract | Arginine (AR) plays an important role in physiological functions, antioxidant status, mitigation of heat stress, and improving performance in birds. This study aimed to evaluate the effect of dietary L-arginine on hematology, protein and lipid profiles, renal, hepatic, and intestinal functions, antioxidant status, and immunity of heat stressed Egyptian geese. A total of 30 sexually mature ganders (local Egyptian male geese strain) with 10 months of age and 3.20±0.25 kg body weight was divided into three groups (10 birds/group). Ganders were kept under normal hot climate in summer of Egypt and fed ad libitum on a commercial mash diet (15.2% CP and ME of 2690 Kcal/kg). The control group (G1) was fed on a basal diet, while G2 and G3 were fed the basal diet with 0.2 and 0.4 g AR/kg, respectively. At the end of an experimental period of three months, five ganders were slaughtered for blood collection and histological study. Hematological and plasma biochemical parameters, liver and kidney function, and antioxidant markers were determined. Results showed that RBCs and lymphocyte increased, while neutrophils and Neut./Lymph ratio decreased in G2, however, Hb, PCV, RBCs, MCH, and lymphocyte increased, while WBCs, neutrophils, and Neut./Lymph ratio decreased in G3 in comparison with G1. Plasma total protein and albumin increased, while total cholesterol, triglycerides, LDL, urea, creatinine, AST, and ALT decreased in G2 and G3 compared with G1. The impaired effects of heat stress on liver and intestine were restored in G3. Plasma total antioxidant capacity, activity of SOD and catalase, and IgG and IgM increased, while malondialdehyde and cortisol decreased in G2 and G3 compared with G1. In conclusion, dietary supplementation of L-arginine (0.4 g/kg) for three months improved hematology, protein metabolism, lipid profile, liver, kidney, and intestine functions, antioxidant status, immunity and welfare of Egyptian geese under heat stress condition.

Keywords | Arginine, Dietary additives, Heat-stressed Egyptian geese, Histology, Antioxidant, Immunity

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***Correspondence** | Eman Said El-Hadad, Animal Production Research Institute, Agricultural Research Center, Dokki, Giza 12618, Egypt; **Email:** dremanhadad@hotmail.com
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INTRODUCTION

Nutritional status has a direct effect on the productivity of farm animals and some nutrients play an important role in controlling the physiological functions of animals (Al-Dabbas *et al*., 2008). Amino acid such as arginine (AR) has a positive impact on antioxidant status (Wu *et al*., 2004) and immunity (Wu and Meininger, 2002) of animals. Both D-arginine and L-arginine play important roles in physiological functions, producing nitric oxide (NO) and polyamines in animal body (Kwon *et al*., 2003), and participate in insulin, glucagon, growth hormone, anti-diuretic hormone synthesis (Soeters *et al*., 2002). The AR administrations have impacts on heart, lung, kidney,

gastrointestinal tract, and liver functions, and also improve animal immune response (Wu *et al*., 2009).

In poultry, as uricotelic species, AR promotes cell division, hormones secretion, and has different physiological roles (Yuan *et al*., 2016), and due to it incomplete urea cycle it cannot synthesize AR (D'Amato and Humphrey, 2010). Poultry requires to a dietary supplementation of AR due to insufficient AR synthesis enzymes (Khajali *et al*., 2013). In laying hens, AR supplements in the diet increased blood NO (Uyanga *et al*., 2022) and improved the growth and feed efficiency (Najib and Basiouni, 2004; Silva *et al*., 2012). Also, dietary AR increased the activity of antioxidant enzymes in rats (Huang *et al*., 2009) and immunity in broilers (Perez-Carbajal *et al*., 2010). In broiler chickens, AR had important role in production of nitric oxide that affects the immune response (Kidd *et al*., 2001) and high AR level can increase antibody production. In geese, dietary supplementation of AR improved protein metabolism, antioxidant enzyme activity, lipid profile, and lipid peroxidation (Chen *et al*., 2023). Moreover, AR can improve hematological parameters in laying hens (Al-Hassani, 2011) and broiler chicken (Al-Daraji and Salih, 2012).

Under the normal condition the requirements of AR in the diet near the NRC recommendation could support the immune system functions. Under heat stress condition in Egypt, the physiological performance of birds was negatively affected by increasing ambient temperature and relative humidity in summer months. The available information about the impaired effects of heat stress or the effect of AR on physiological performance and histological changes in Egyptian geese is rare. Some reports explored the role of AR in eliminating the negative effects of heat stress (Tong and Barbul, 2004).

Therefore, the objective of the current study was to study the possibility of using arginine as a dietary supplementation for Egyptian geese to mitigate heat stress by improving the hematological, protein, and lipid profiles, hepatic and intestinal histogenesis, antioxidant status, and immunity.

MATERIALS AND METHODS

This experiment was carried out at El-Serw Research Station (Located in North East of the Nile Delta. Dumitta Governorate, 32 latitude N;zero altitude), belonging to Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agricultural and Land Reclamation during the interval from May to July. All handling and management processes of birds in this study were done in accordance with the Directive 2010/63/EU for animal protection that used for scientific purposes (Official Journal of the European Union, 2010).

Climatic condition

During the experimental period, surrounding ambient temperature (AT) and relative humidity (RH) were recorded to calculate values of temperature-humidity index (THI). Averages ± SD of AT, RH, and THI were 37.72±3.94 °C, 73.44±5.38%, and 93.78, respectively. Throughout the experimental period, the natural photoperiod ranged between 13-14 h light and 10-11 h dark. According to the equation of LPHSI (1990), THI = db - (0.55 - 0.55 RH) (db – 58) Where: db = dry bulb temperature (°F), RH = relative humidity/100. THI values of 72-79 indicate mild heat stress, 79-89 moderate heat stress, and >89 severe heat stress. This indicates that all groups of geese were kept under heat sever stress conditions during the experimental period. Ganders were kept under the natural light during the daytime.

Birds

A total number of 30 sexually mature ganders (local Egyptian male geese strain) at 10 months old and averaged 3.20±0.25 kg body weight was used in this study. The experimental birds were divided into three groups (10 birds/group). Ganders were kept in an intensive system in windowless houses with ten pens (2.5 x 2.5 m^2) which were furnished with wood shaving as litter. The experimental ganders were fed *ad libitum* on a commercial mash diet (15.2% CP and ME of 2690 Kcal/kg) for two months as an experimental period. Drinking water was provided *ad-libitum*. Ingredient of the basal diet is present in Table 1.

Table 1: Ingredients of the basal diet.

Feedstuff	%	Feedstuff	%
Yellow corn	63.00	Calcium carbonate (Ca Co3)	1.80
Soya bean meal (44%)	15.50	Vitamin & minerals mixture	0.30
Wheat bran	17.78	Sodium chloride (Na Cl)	0.30
Di-caphos.	1.25	D.L. Methionine	0.07
Total		100	

Experimental groups

Three experimental groups in this study 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 (99% L-Arginine; HSN: 2922.4990, CAS No: 74-79-3: $C_6H_{14}N_4O_2$, M.W. 174.20, Loba chemie pvt. Ltd., Mumbai, India) per kg diet, respectively. At the end of an experimental period of three months, five ganders were slaughtered for blood collection and histological study.

Evaluating some blood constituents

At the end of the experiment, blood samples were collected at slaughter from 5 birds in each group into heparinized tubes. In whole blood samples, hematological parameters

including concentration of hemoglobin, packed cell volume (PCV), red cellular number (RBCs) and white cellular (WBCs) number, mean corpuscular of hemoglobin (MCH), volume (MCV), and hemoglobin concentration (MCHC) as well as percentage of neutrophils and lymphocytes were determined by using a veterinary hematology analyzer (Exigo, Boule medical AB., Sweden) according to Helper (1977), Lucas and Jamroz (1961), Eilers (1967), and Wintrobe (1981), respectively.

Blood samples were centrifuged at 3000 rpm for 15 minutes to obtain blood plasma which was kept at -15°C until the chemical analysis. Plasma concentrations of total protein, and albumin were estimated according to Henry (1964). However, concentration of total cholesterol, triglycerides, high density lipoprotein (HDL), urea, and creatinine were determined after the methosds described by Doumas *et al.* (1997), Richmond (1973), McGowan *et al.* (1983), Tietz (1987) and Bartles *et al.* (1972), respectively. All blood biochemical were assayed by spectrophotometer using commercial medical kits (Nanjing Jiancheng Biochemical Reagent Co., China). However, globulin concentration was calculated (Globulin= total protein – albumin). Activities of transaminases (AST and ALT, IU/L) were estimated in blood plasma according to Reitman and Frankel (1957). Levels of total antioxidant capacity (TAC), (Erel, 2004) and malondialdehyde (MDA), Richard *et al.* (1992) as well as superoxide dismutase (SOD), Nishikimi *et al.* (1972) and catalase (Aebi, 1984) in blood plasma were also measured. ELISA kits (Kamiya Biomedical Company, USA) was used for assaying immunoglobulin concentration of IgG and IgM. Cortisol concentration was determined in blood plasma by Nichols and Nelson (1977).

Histological study

At the end of the experimental period, five ganders from each group were slaughtered, then liver and intestine each gander was isolated from each carcass. The histological samples were taken from the median hepatic lobule and the media region of the small intestine. According to Fischer *et al.* (2008), small specimens were taken 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. The histological examination was performed using microscope (Swift SW350T) at x400 magnification.

Statistical analysis

Analyses of variance was done by the least square analysis of variance utilizing the General Linear Model Procedure (SAS, 2013). The model was as follows: $Y_{ij}= \mu + T_i + e_{ij}$ Where Y_{ij}=any observation of ith bird within j^{th} treatment, µ= overall mean, T_i= effect of i^{th} treatment (i:1-4), e_{ij}= random error. Multiple range test (Duncan, 1955) was used to separate the significant differences among means.

RESULTS AND DISCUSSION

Hematological profile

Results in Table 2 show that RBCs count and lymphocyte percent, significantly increased, while neutrophils percent and Neut./Lymph ratio in blood of ganders were decreased by low AR level (G2) as compared to control (G1). However, the higher level of AR (G3) significantly increased Hb concentration, PCV, RBCs count, MCH value, and lymphocyte percent, and significantly decreased WBCs count, neutrophils percent, and Neut./Lymph ratio in comparison with control (G1). However, MCV and MCHC values were not affected significantly by AR supplementation. In accordance with our results in goose, Al-Daraji and Salih (2012) reported that supplementation of dietary AR at a level of 0.06% improved hematological parameters such as RBCs, Hb, PCV, and MCH in blood of broiler chicken.

Table 2: Effect of dietary arginine supplementation on hematological parameters of ganders.

Hematological parameters	G1 (Control)	G2 (0.2 g AR)	G3 (0.4 g AR)	SEM	P value
Hemoglobin (g/dl)	12.03^{b}	12.16^{b}	14.10^{a}	0.30	0.0048
PCV (%)	40.73^{b}	40.10^{b}	45.36^{a}	0.47	0.0004
Erythrogram indices					
Red blood cells (10^6/mm^3)	3.40^{c}	4.04^{b}	4.73^{a}	0.05	0.0001
MCH (pg/cell)	67.46^{b}	69.70^{ab}	74.00^{a}	1.42	0.0450
MCV (FL/cell)	166.53	171.13	172.1	3.93	0.5926
MCHC (g/dl)	41.97	42.5	42.13	0.67	0.8527
Leukogram indices					
White blood cells (10^3/mm^3)	8.36^{a}	7.86^{ab}	7.12^{b}	3.43	0.0106
Neutrophils (%)	26.00^{a}	20.13^{b}	18.33^{c}	1.01	0.0041
Lymphocytes (%)	58.20^{c}	64.03^{b}	73.10^{a}	1.71	0.0024
Neut./Lymph. ratio	0.44^{a}	0.31^{b}	0.25^{c}	0.01	0.0001

$^{a, b, and c}$: Means, within each row with different superscripts are significantly different at P<0.05.

In accordance with our results in goose, dietary AR (0.06%) improved RBCs, Hb, PCV, and MCH in blood of broiler chicken (Al-Daraji and Salih, 2012). In laying hens, WBCs count was reported to improve significantly by dietary AR as compared to controls under the normal condition (Al-Hassani, 2011). The observed reduction in WBCs count especially in G3 supplemented with the highest AR level may be due to improving immune response and antioxidant status by AR treatment versus undergoing controls to

heat stress. This was proved by increasing percentage of lymphocytes significantly in treatment groups (G2 and G3) compared with control (G1). Also, Liu *et al.* (2019) reported that AR administration increased the pre-B lymphocytes differentiation, then B lymphocytes was released in bone marrow that promote secretion of immunoglobulins. Moreover, dietary AR (0.30 and 0.60%) can stimulate the lymphocyte proliferation in broilers (Perez-Carbajal *et al.*, 2010).

Protein metabolites

Protein metabolism in terms of concentration of total protein and albumin in blood plasma of ganders were significantly increased by high AR level (G3) only. However, AR supplementation failed to affect plasma globulin concentration or albumin/globulin ratio (Table 3). These results indicated that AR treatment increased plasma total protein in ganders, in terms of increasing albumin, not globulin concentration.

Table 3: Effect of dietary arginine supplementation on total protein and their fractions in blood plasma of ganders.

Protein metabolites	G1 (Control)	G2 (0.2 g AR)	G3 (0.4 g AR)	SEM	P value
Total protein (g/dl)	5.40[b]	5.85[b]	6.40[a]	0.07	0.0002
Albumin (g/dl)	3.15[c]	3.45[b]	3.85[a]	0.05	0.0003
Globulin (g/dl)	2.25	2.40	2.55	0.11	0.2738
Albumin/globulin ratio	1.40	1.44	1.51	0.09	0.1437

[a, b, and c]: Significant group differences at P<0.05.

In agreement with our results, blood total protein concentration significantly increased by dietary AR in geese (Chen *et al.*, 2023) and in rabbits (Ahmed, 2021). In this context, AR is a major source of N in the body which activates the protein synthesis by stimulation of amino acid-sensitive targets, then effecting on level of protein in the blood (Alimohammadi *et al.*, 2015).

Lipid profile

Lipid profile including concentration of total cholesterol, triglyceride, and LDL in blood plasma of ganders in G2 and G3 were decreased (P<0.05) by both AR levels in comparison with G1 (Table 4).

Table 4: Effect of dietary arginine supplementation on lipid profile in blood plasma of ganders.

Lipid type	G1 (Control)	G2 (0.2 g AR)	G3 (0.4 g AR)	SEM	P value
Total cholesterol (mg/dl)	183.0[a]	162.5[b]	157.5[c]	2.04	0.0002
Triglycerides (mg/dl)	111.5[a]	92.0[b]	93.0[b]	2.11	0.0010
LDL (mg/dl)	39.50[a]	35.50[b]	33.00[c]	0.62	0.0009

[a, b, and c]: Means, within each row with different superscripts are significantly different at P<0.05.

Our results indicated an important role for AR in improving lipid metabolism by regulation of lipid profile in blood plasma of ganders. In agreement with our results. Chen *et al.* (2023) found that the concentration of triglyceride in the blood of geese was reduced by AR supplementation. In broiler chickens, Fouad *et al.* (2013) observed that blood concentration of triglycerides significantly reduced by dietary AR supplementation (0.25 or 1.00%) as compared to controls. This result was proved also in Nile tilapia fish by Li *et al.* (2020), who observed that dietary AR (1 and 2%) decreased level of triglycerides in the blood with marked reduction in lipid accumulation in liver.

The reduction in level of plasma triglycerides in G2 and G3 may be due to that: (1) AR administration regulates lipid metabolism by the control on fatty acid synthesis and related metabolism enzymes (Li *et al.* 2020), and (2) NO produced by AR metabolism regulates acetyl-CoA carboxylase activity and inhibits fatty acid synthesis as mentioned by García-Villafranca *et al.* (2003). These finding indicated that AR administration reduced the metabolic diseases risk of ganders by improving plasma lipid profile.

Kidney function

Plasma concentration of urea and creatinine, were significantly lower in treatment groups (G2 and G3) than in control one (G1), but the lowest values of urea and creatinine were significantly recorded in G3 (Table 5). These results indicated beneficial effects of dietary AR supplementation on kidney function of ganders under heat stress condition. The vital role of AR treatment on kidney function was reported by Wu *et al.* (2009).

Table 5: Effect of dietary arginine supplementation on kidney function markers in blood plasma of ganders.

Plasma biochemicals	G1 (Control)	G2 (0.2 g AR)	G3 (0.4 g AR)	SEM	P value
Urea (mg/dl)	29.50[a]	26.50[b]	21.50[c]	0.86	0.0017
Creatinine (mg/dl)	1.35[a]	1.17[b]	1.15[b]	0.02	0.0023

[a, b, and c]: Means, within each row with different superscripts are significantly different at P<0.05.

In heat stressed broilers, blood level of uric acid increased in the blood (Kataria et al., 2008; Dao *et al.*, 2021) due to a reduction in digestion of nitrogen with more degradation of nitrogen to uric acid due to cyclic warm temperature. This was proved in ganders fed diet without AR supplementation. In this context, Hilliar et al. (2019) found more efficient use of the dietary nitrogen and low uric acid level produced by birds fed AR diets, and with potential benefit in heat stressed birds.

Liver function

Liver enzyme activity

In blood plasma of ganders, activity of AST and ALT, as liver function parameter, was reduced (P<0.05) due to AR supplementation either at low or high level. However, AST/ALT ratio was not affected by AR treatment (Table 6).

Table 6: Effect of dietary arginine supplementation on activity of transaminases (AST and ALT) in blood plasma of ganders.

Activity	G1 (Control)	G2 (0.2 g AR)	G3 (0.4 g AR)	SEM	P value
AST (U/L)	81.50[a]	65.00[b]	61.00[c]	2.41	0.0021
ALT (U/L)	19.90[a]	17.85[b]	16.70[b]	0.57	0.0208
AST/ALT ratio	4.10	3.64	3.65	0.13	0.1025

[a, b, and c]: Significant group differences at P<0.05.

Hepatic histological structure

The histological examination of gander livers in the control group (G1) revealed impaired effects of heat stress on the normality of hepatic architecture. Liver of ganders in the control group showed extensive perivascular leukocytes aggregations infiltrating the hepatic parenchyma (Figure 1A). Marked perivascular hepatic necrosis was also seen in terms of complete loss of hepatic architecture surrounded and invaded with lymphocytes admixed with few macrophages and fibroblasts (Figure 1B). The impaired effects of heat stress on liver histology may be in association with oxidative stress under heat stress conditions.

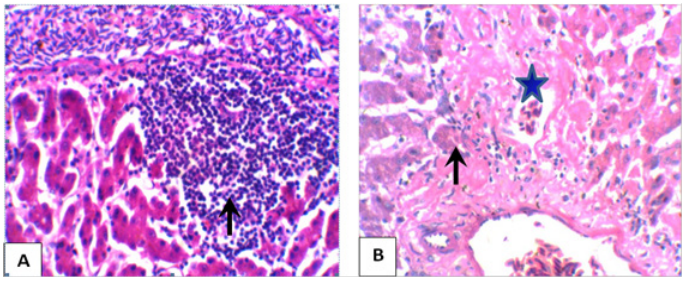

Figure 1: Representative photomicrograph of gander livers in the control group showing: (A) extensive perivascular leukocytes aggregations infiltrating the hepatic parenchyma (arrow), and (B) marked perivascular hepatic necrosis (star) and lymphocytes admixed with few macrophages and fibroblasts (arrow). (H & E, x400).

Livers of ganders fed diet with low AR level (G2) showed mild impact on improving the histological structure of the liver, in terms of perivascular focal to coalescing mild lymphocytic aggregations admixed with macrophages (Figure 2).

Increasing AR level in G3 showed restoration of most hepatic architecture with few occasional perivascular lymphocytes, leading to normal architecture of the hepatic lobules with intact arranged hepatocytes and hepatic veins (Figure 3). These findings suggest that the higher AR level may aluminate the impaired effects of heat stress on ganders.

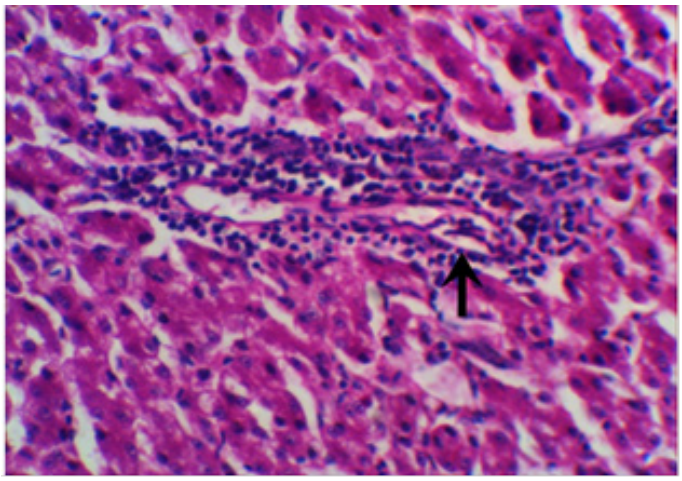

Figure 2: Representative photomicrograph of gander liver in G2 showing perivascular focal to coalescing mild lymphocytic aggregations admixed with macrophages (arrow). (H & E, x400)

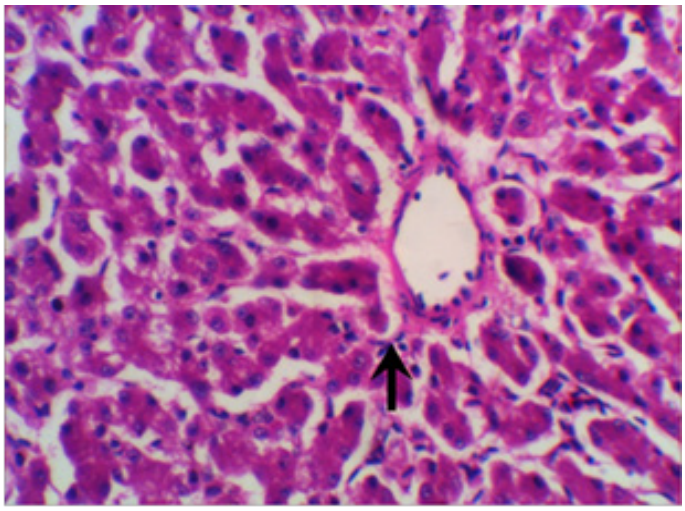

Figure 3: Representative photomicrograph of gander liver in G3 showing normal architecture and intact hepatocyte restoration of most hepatic architecture with few occasional perivascular lymphocytes (arrow). (H&E, x400).

The reduction in activity of plasma AST and ALT, being significantly lower with high than with low AR level as well as the histological improvements in the hepatic architecture of ganders, being mild with low AR level and the best with high AR level, indicated the beneficial impacts of dietary AR supplementation on a level of 0.4 g/kg on restoring the normal liver function of heat stressed ganders as reported by Wu *et al*. (2009).

Unfortunately, there are no information of the AR effect on the histological structure of the liver in goose. In

contrast to our results, Alabi *et al.* (2018) observed that the liver of chickens was negatively affected by high or low AR level. AR supplementation at a level of 167 mg/L showed congested vascular spaces, while the liver in those supplemented with 334 mg/L showed congested vascular spaces and periportal mononuclear inflammatory infiltration as examined by Pacher *et al.* (2007).

Intestinal histology

The histological examination of the gander intestine in the control group (G1) revealed that exposure to heat stress resulted in gander intestine to show focal cellular infiltrates expanded to separate the intestinal crypts (Figure 4A) and multifocal expanded to separate the intestinal crypts and tunica submucosa (Figure 4B). These focal and multifocal were characterized by mucosal and muscular mass aggregations of cellular infiltrates, clearing extensive granulomatous enteritis.

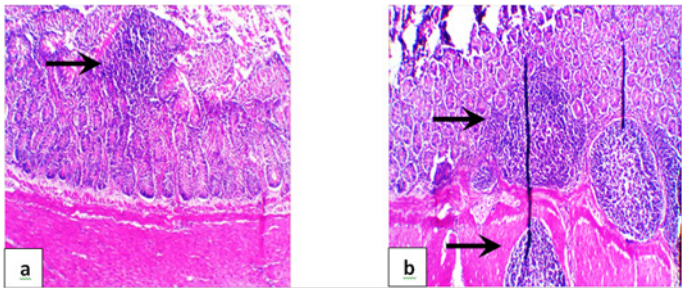

Figure 4: Representative photomicrograph of gander intestine in control group (G1) showing focal of cellular infiltrates (a & b) in the intestinal crypts to multifocal of cellular infiltrates (b) in the intestinal crypts and tunic submucosa. (H & E, x400).

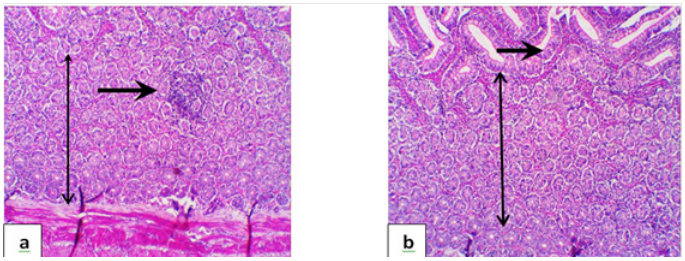

Figure 5: Representative photomicrograph of gander intestine in G2 showing focal of mild cellular infiltrates (a), villus fusion with thickness of villous lamina epithelialis mucosa, extensive numerous intestinal crypt proliferation, and few cellular infiltrates. (H & E, x100).

In gander of G2 fed low AR level showed slight proliferative enteritis represented by focal mild cellular infiltrates separated a marked proliferative crypt (Figure 5A), villus fusion with thickness in of villous lamina epithelialis mucosa, extensive numerous intestinal crypt proliferation, and few or no cellular infiltrates (Figure 5B). These observations revealed that gander fed low AR level exhibited slight enteritis.

Increasing the level of AR in the diet of gander in G3 mitigated the incidence of enteritis and intestine of ganders showed focal apical villus sloughing with mild cellular infiltrates and mild to moderate intestinal crypt proliferation (Figure 6A). Also, AR treatment resulted in restoration or regeneration of most intestinal architecture (Figure 6B).

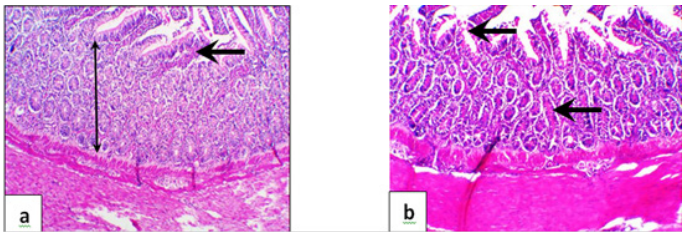

Figure 6: Representative photomicrograph of gander intestine in G3 showing focal apical villus sloughing (arrow) with mild cellular infiltrates and mild to moderate crypt proliferation (a) and intactness of most intestinal architecture (b). (H & E, x100).

The histological examination indicated impaired effects in heat stressed gander in the control group, eliminated to mild negative effects by low level of AR treatment, and improved by high AR level in the diet of gander. In this respect, Wu *et al.* (2009) reported that dietary AR supplementation was suggested to have positive impact on gastrointestinal tract.

A normal structure of the small intestinal mucosa is necessary for optimal growth as well as nutrient digestion and absorption (Yao *et al.*, 2012). One of the characteristics of the intestinal villus epithelium is a short proliferation cycle and rapid growth (Sigdestad and Lesher, 1970; Cheng and Leblond, 1974). Villus height and crypt depth affect the efficiency of the intestinal absorptive area. Heat stress has pathological damage on the duodenum, jejunum and ileum, particularly epithelial cells and villus size (Zhangyong *et al.*, 2003). A mild improvement in the intestinal architecture by low AR level and higher improvement by high AR level in heat stressed ganders were observed in our study. AR, as a precursor of polyamines, may stimulate the intestinal mucosa development by acceleration of the mitotic division in the villus-crypt layer (Murakami *et al.*, 2012). Also, AR and its product NO have a role in stimulation of the cell proliferation of the lamina epithelialis (Marc Rhoads and Wu, 2009). Dietary AR improved gut morphology and intestinal nutrient absorption by producing polyamines, increasing cellular mitosis, and/or activation of NO (Khajali et al., 2014; Abdulkarimi et al., 2019). Dietary AR (1.44 or 1.69%) increased height of the villus and villus: crypt ratio, but increasing AR level to 2.19% decreased villus length and height of crypts (Yu *et al.*, 2018). Additionally, some authors reported an improvement in intestinal permeability by AR (Costa *et al.*, 2014; Castro *et al.*, 2020). Finally, AR has the ability to stimulate the development

of the intestine (Nie *et al*., 2018; Martí and Reith, 2021).

Lipid peroxidation and antioxidative status

Administration of AR at low or high level improved lipid peroxidation by increasing (P<0.05) plasma total antioxidant capacity and activity of antioxidant enzymes (SOD and catalase), and decreasing (P<0.05) plasma malondialdehyde level. Increasing level of AR from 0.2 to 0.4 g/kg showed more beneficial effects on lipid peroxidation and antioxidant enzyme activity (Table 7).

Table 7: Effect of dietary arginine supplementation on lipid peroxidation, antioxidant enzymes, immunoglobulins, and cortisol in blood plasma of ganders.

Parameter	G1 (Control)	G2 (0.2 g AR)	G3 (0.4 g AR)	SEM	P value
Lipid peroxidation parameters					
TAC (mmol/l)	27.65^{c}	32.65^{b}	36.25^{a}	0.49	0.0001
MDA (nmol/ml)	18.65^{a}	15.35^{b}	13.15^{c}	0.22	0.0001
Antioxidant enzyme					
SOD (mg/dl)	36.90^{c}	40.65^{b}	47.85^{a}	1.21	0.0018
Catalase (mg/dl)	228.25^{c}	256.10^{b}	300.50^{a}	5.51	0.0002

$^{a, b, and\ c}$: Means, within each row with different superscripts are significantly different at P<0.05.

The antioxidant defense system in the body included TAC, SOD, and catalase. Lipid peroxidation incidence and the oxidative damage were reflected by MDA level (Luo *et al*., 2011). SOD has an important role in the protection of the cells from H_2O_2 damage, and promotes the resistance of disease (Maksimenko, 2005). As reported in our study, SOD activity was improved and MDA level was decreased, while, TAC was not affected by AR treatment in geese (Chen *et al*., 2023). The activity of antioxidant enzymes was increased and MDA level was decreased in blood of rats (Liang *et al*., 2018) and geese (Chen *et al*., 2023) as affected by dietary AR. Also, AR produce NO which decrease oxidative stress by elevating activity of SOD and decreasing MDA level (Dasgupta *et al*., 2006). The high AR level may suppress the antioxidant activity, and reduces excessive free radical the generation which induces lipid peroxidation then cellular damage (Chen *et al*., 2023).

Table 8: Effect of dietary arginine supplementation on immunoglobulin concentration in blood plasma of gander.

Parameter	G1 (Control)	G2 (0.2 g AR)	G3 (0.4 g AR)	SEM	P value
Immune response					
IgG (mg/dl)	30.35^{c}	36.55^{b}	40.90^{a}	0.94	0.0006
IgM (mg/dl)	14.05^{c}	20.20^{b}	27.90^{a}	0.81	0.0001

$^{a, b, and\ c}$: Means, within each row with different superscripts are significantly different at P<0.05.

Immune response

Results in Table 8 show that, both AR levels improved plasma immunoglobulins (IgG and IgM) concentration.

In birds, resistance to diseases can reflected by blood immunoglobulin levels which are antibodies having specific binds with antigens (Wu *et al*., 2021; Chen *et al*., 2023). IgG has activities against bacteria and viruses to regulate the immune function by agglutinating with antigen and then precipitating it (Wu *et al*., 2021), so IgG levels can reflect the bird health status and dietary AR improves animal immunity (Wu *et al*., 2009). In our study, AR treatment increased plasma IgG and IgM levels as found by Chen *et al*. (2023) in goose treated with AR (0.3%). In broilers, an increase in serum IgM and IgG concentrations occurred by dietary AR (0.30 and 0.60%) as reported by Liu *et al*. (2019).

AR has an important role in production of NO that influences the immune system of birds under different conditions (Kidd *et al*., 2001). NO-synthase can catalyze AR to produce NO, leading to an improvement in immune response (Birmani *et al*., 2019). In this respect, Uyanga *et al*. (2022) reported a linear response for blood NO level in consistent with AR treatment. A high AR level can force antibody production in broiler chickens (Abdukalykova and Ruiz-Feria, 2006). AR can increase the function of immune system and improve the size of thymus gland (Al-Daraji and Salih, 2012). Also, AR had important role in production of immunoglobulins from metabolites by arginase, and is important for the development of Lymphocyte (Fathi *et al*., 2017) as proved in our study.

Stress status

Treatment with AR at a level of 0.2 g/kg reduced (P<0.5) plasma concentration of stress hormone cortisol. Increasing level of AR to 0.4 g/kg exhibited further decrease in plasma cortisol level of ganders in G3 (Figure 7).

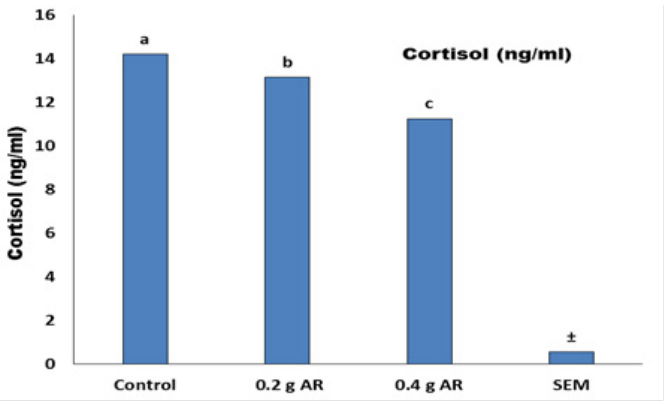

Figure 7: Effect of dietary arginine supplementation on lipid peroxidation biomarkers, activity of antioxidant enzymes, immunoglobulins, and cortisol in blood plasma of geese at the end of experiment.

In broilers and chickens, concentration of cortisol significantly elevated under heat stress condition as reported by Soleimani *et al.* (2011) and Zhang *et al.* (2019), respectively. Under stress condition, hypothalamic-pituitary-adrenal (HPA) axis was stimulated in terms of increasing cortisol level (Eutamene and Bueno, 2007). The reduction in cortisol level is important for the rise in thyroxin concentration in the supplemented groups because elevated concentrations of adrenal cortical hormones are considered to be responsible for hypothyroid activity (Ganong, 2018). In agreement with our results, serum cortisol level was reduced by amino acid mixture in the diet of piglets compared with the controls (Prates *et al.*, 2021). In contrast, Sohail *et al.* (2010) showed an opposite trend in Hubbard chicks. Based on the previous findings, AR has a role in reducing the stress especially in heat stress (Tong and Barbul, 2004).

CONCLUSION AND RECOMMENDATION

Based on the foregoing results, dietary supplementation of arginine at levels of 0.4 g/kg for three months under heat stress condition increased erythrogram and leukogram indices (Lymphocytes), and plasma proteins, improved plasma lipid profile by reducing total cholesterol, triglycerides, and LDL, maintained liver, kidney, and intestine functions, and enhanced antioxidant status, welfare, and immunity of geese under heat-stress condition in Egypt. The current study recommended L-arginine to be as a dietary additive to improve performance of heat-stressed geese.

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NOVELTY STATEMENT

L-arginine can use in Egyptian male geese treatment as feed additive to mitigate the undesirable effects of heat stress condition during summer season in Egyptian.

AUTHOR'S CONTRIBUTION

All authors were suggested the experimental design and achievement of the experimental work. MAEH and AME-Shhat were conducted the experimental procedures and collected data. HAM, MFS performed the sample preparations and chemical analysis. ESE-H and EZE conducted the statistical analyses and critically revised the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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