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REPRODUCTIVE PERFORMANCE, OXIDATIVE STATUS AND BLOOD METABOLITES OF DOE RABBITS ADMINISTRATED WITH SPIRULINA ALGA

El-Ratel, I.T.

Dep. of Poult. Prod., Fac. of Agric., Damietta Uni., Damietta, Egypt.

Corresponding Author: ibrahim.talat81@yahoo.com

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ABSTRACT: The current study aimed to evaluate effects of oral administration with different levels of Spirulina platensis on reproductive performance, hematological and biochemical criteria, antioxidant activity, and liver and kidney histogenesis of APRI doe rabbits. A total of 45 nili-parous does at 16-18 wk of age was allotted to 3 groups (15 does / group). Does in the 1st, 2nd and 3rd groups were daily received oral 3 ml distilled water containing 0 (G1) as control group, 300 (G2) and 600 (G3) mg/doe from Spirulina platensis for four weeks as a treatment period, respectively. At end of treatment, does were naturally mated by15 untreated fertile APRI bucks (5 bucks / group). Body weight (BW) of does, as initial and final of treatment (16-18 and 20-22 wk of age, respectively) and at kindling (22-26 wk of age), and of bunnies at birth and weaning were recorded for one parity. Daily feed intake (DFI) of does was recorded during treatment period. Conception (CR) and kindling (KR) rates and litter size (LS, total and live) of does, and bunny viability rate (VR) at birth and weaning were calculated. Plasma concentration of estradiol 17-β (E2) and progesterone (P4) of does on day 15 post-mating, hematological parameters, plasma biochemical and oxidative capacity at the end of treatment, and hepatic and renal histology of does at the end of experiment were determined.

Results showed that doe final BW was heavier (P<0.05) in G2 than in G1 and G3. Change in BW (absolute or relative to initial BW), DFI, CR and LS of does, and VR and BW at birth and weaning of kids increased (P<0.05) in G2 and G3 than in G1, but KR was similar. Plasma E2 and P4 was higher (P<0.05), while E2/P4 ratio was lower (P<0.05) in G2 and G3 than in G1.

Only E2 was higher (P<0.05) in G2 than in G3. Values of hemoglobin, red blood cells hematocrit, neutrophils and eosinophils increased (P<0.05) in G2 and G3, while platelets and lymphocytes, monocytes and acidophils decreased (P<0.05) in G2 and G3 compared with G1. The white blood cells decreased (P<0.05) only in G2, while erthrocytic values were not affected. Plasma total proteins, albumin, globulin and high density lipoprotein increased (P<0.05) in G2 and G3, while total lipids, triglycerides, total cholesterol and low density lipoprotein, aspartate aminotransferase, alanine aminotransferase, acid phosphatase and alkaline phosphatase decreased (P<0.05) in G2 and G3 compared with G1. Blood total antioxidant capacity, glutathione S-transferase, superoxide dismutase, glutathione and glutathione peroxidase increased (P<0.05), while thiobarbituric acid-reactive substances decreased (P<0.05) in G2 and G3 than in G1. Hepatic and renal tissues of treated does were normal.

In conclusion, orally administrated Spirulina platensis (300 mg/doe) as natural antioxidants was proved to improve reproductive performance, blood constituents, antioxidative status without adverse effects on liver and kidney functions.

Key words: Rabbit, antioxidants, reproduction, peroxidation, histology, liver, kidney.

INTRODUCTION

In comparing with other livestocks, rabbit has a great attention due to their superiority in white meat yield, productive and reproductive performances, and economic efficiency, beside short pregnancy length (Basavaraj et al., 2011). In recent years, to improve the genetic selection and reproductive management, commercial rabbit breeder profitability has increased (Sirotkin et al., 2014).

During cell metabolism, normal production of oxidants is indispensable for the regulation of redox in the cell (Kobayashi et al., 2001) to support the microorganisms phagocytosis (Castellini et al., 2000). Several physiological processes, including steroidogenesis in the ovaries, maturation and fertilization of oocytes, implantation, maintenance of CLs during pregnancy and parturition in rabbits (Abdel-Khalek et al., 2008) and human (Sekhon et al., 2010) were found to be affected by antioxidant system.

Recently, there are great attentions toward the usage of natural active compounds, namely microalgae as an addition to diet or water to improve rabbit reproduction without adverse effects on animal health (Ragab and Abd El-Lateif, 2016; Fouda and Ismail, 2017). Microalgae are microorganisms, which are classified into Spirulina platensis, S. fusiformis and S. maxima (Karkos et al., 2011).

Spirulina alga has high poly-nutrients value and phytopigments in a simple structure with a complex composition (Abu-Elala et al., 2016). It contains vital compounds, such as protein (50-70% on DM basis) with all essential amino acids (Farag et al., 2016), essential fatty acids, alpha-linolenic, gamma-linolenic and linoleic (Mendes et al., 2003), photosynthetic pigments (Bermejo et al., 2008), vitamins such as thiamine, nicotinamide, riboflavin, folic acid, pyridoxine, vitamins A, D and E vitamins (Hoseini et al., 2013) and minerals like Ca, K, Cr, Cu, Mn, Fe, P, Mg, Na, Zn and Se (Babadzhanov et al., 2004). Therefore, Spirulina has highly international demands and is considered as safe and healthy food and animal feed for therapeutic practices (Vonshak and Tomaselli, 2000).

Oxidative processes as a result of exposing to metabolic, environmental or nutritional stressors affect normal cell functions, initiate chain reactions that can compromise cell integrity (Lykkesfeldt and Svendsen, 2007).

Under different stressors, antioxidants have the ability to prevent cell damage in antioxidant defense system by counteracting the oxidants and other cellular protection (Mittler et al., 2004). In rabbits, several natural antioxidants are playing an important role in improving animal reproduction, especially embryo implant, placenta formation, and growth of fetuses, decreasing lipid profile and improving feed conversion rate (El-Ratel et al., 2017). In this respect, Spirulina is considered as a strong natural antioxidant, having anti-lipid peroxidation (Kurd and Samavati, 2015).

In addition, spirulina contains both enzymatic (superoxide dismutase, catalase and glutathione peroxidase, peroxiredoxin and ascorbate peroxidase) and non-enzymatic (carotenoids, tocopherols, ascorbic acid, glutathione and chlorophy-ll derivatives) in antioxidant defense system, which

remove oxidants to protect the cells from harmful effects of stress conditions (Abd El-Baky et al., 2007). Also, Spirulina was reported to reduce the toxicity and improve digestibility and palatability (Abdel-Daim et al., 2013). Based on previous studies, dietary supplementation with Spirulina had beneficial effects on productive and reproductive performance, good health status and more economic efficiency of animals and poultry compared with other chemical products (El-Sabagh et al., 2014; Shanmugapriya et al., 2015). Previous reports mentioned that algae treatment may be ascribed to dose and type of the administration, kind of algae, animal species and/or treatment period.

Therefore, the objective of this study was to investigate the effect of oral administration with different levels of Spirulina platensis (0, 300 and 600 mg/doe) for four weeks pre-mating on reproductive performance, hematological and biochemical criteria, antioxidant activity and liver and kidney histogenesis of doe rabbits (APRI line).

MATERIALS AND METHODS

This study was carried out at a private rabbit farm, located in Tanikh Village, Nabroh City, Dakahlia Governorate, Egypt, while laboratorial work was carried out at Physiology and Biotechnology Laboratory, Animal Production Department, Faculty of Agriculture, Mansoura University, Egypt, during the period from March to June 2017.

Total of 45 APRI nili-parous doe rabbits at 16-18 wk of age were used in this study. Does were similarly divided into 3 groups (15does/group) according to live body weight and parity order. All experimental does were allowed to acclimatize for one week as an adaptation period in their cages. Throughout the experimental period all does were exposed to similar managerial and environmental conditions. Does were housed in individual metal cages prepared with feed suppliers and nipple for drinking water.

Does were fed commercial complete feed pelleted diet (CFD) containing 30% clover hay, 21% wheat bran, 5% yellow corn, 18% soybean meal, 3% molasses, 21% barley grain, 1% limestone, 0.5% common salt, 0.3% premix and 0.2% DL-methionine. The CFD contained 18% CP, 14% CF and 2800 Kcal/kg diet according to NRC (1977) and was offered twice daily.

Doe rabbits in the 1st group were given sterile distilled water (3 ml) and served as control (G1) as oral dose by plastic syringe (5 ml), Does in the 2nd and 3rd groups were received daily 3 ml distilled water supplemented with 300 mg (G2) or 600 mg (G3) of Spirulina platensis for each doe for four weeks as a treatment period. Spirulina platensis in powder form was dissolved in distilled water at a rate of 1 and 2 g per 100 ml distilled water for treatment groups, G2 and G3, respectively. Samples of Spirulina platensis powder (prepared in National Institute of Oceanography and Fisheries, Egypt) were taken for a complete chemical analysis included 85.77% DM, 57.66% CP, 1.75% EE, 12.93% NFE, 3.6% CF and 9.83% ash. Live body weight of does was recorded as initial and final weights at 16-18 and 20-22 wk of age, respectively and at kindling. Bunny

weight at birth and weaning (4 wk of age) was also recorded. Through treatment period of 4 wk, feed intake of does was recorded.

After four weeks of treatment, all does were naturally mated using untreated fertile APRI bucks. For this purpose, total of 15 rabbit bucks were used in this study for natural mating does in experimental groups (5 bucks for each). Bucks were individually housed in cages (similar to that of does, but without nest boxes). Pregnancy was diagnosed by manual palpation to detect conceived does 10-12 days post-mating. Nest boxes were supplied with sawdust for conceived does. Kindling rate was calculated and gestation period length was recorded after kindling. After 12 h of kindling, total and live litter size at birth recorded and stillbirth was removed. Kits were left with their dams and weaned on day 28 of age. Litter size at weaning was determined and viability rate at birth and weaning was calculated. All reproductive performance parameters were recorded during the first parity of does.

After four weeks of treatment (20-22 wk of age, at mating) blood samples were taken (five does in each group) from ear vein into sterile test tubes with EDTA for estimation of concentration of hemoglobin (Hb), hematocrit value (Ht), red (RBCs), white (WBCs) blood cells and platelets counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in whole blood samples were determined according to Wintrobe (1967). Also, leukocyte fraction, including percentage of lymphocytes, monocytes, eosinophils and neutrophils were determined (Lucky, 1977).

The collected blood samples were centrifuged at 3500 rpm for 15 min to separate blood plasma stored at -20^{o}C for biochemical analysis of total proteins, albumin, total lipids, total cholesterol, triglycerides and glucose concentrations, as well as, activity of aspartate (AST), alanine (ALT) aminotransferase, acid (ACP) and alkaline (ALP) phosphatases using commercial kits (Diamond Diagnostics, Egypt) and spectrophotometer. Globulin concentration was obtained by difference.

Oxidative capacity parameters, including activity of total antioxidant capacity (TAC), glutathione content (GSH), glutathione peroxidase (GPx), glutathione S-transferase (GST), superoxide dismutase (SOD) and thiobarbituric acid-reactive substances (TBARS) were assayed in blood plasma using commercially available kits (Bio Diagnostic Research).

On the other hand, other blood samples were collected on day 15 post-mating for determination of estradiol-17β (E2) and progesterone (P4) concentration in 5 conceived does in each group. Hormonal concentration was assayed by radioimmunoassay (RIA) using DSL-43100 and DSL-3900, respectively (Diagnostic systems Laboratories Inc, TX, USA) according to Abraham (1977).

Does were sacrificed (3 from each group) at weaning (end of experiment, at 28-30 wk of age). Small specimens from tissues of liver and kidney were taken for fixation for 24 h (10% neutral buffer formalin solution), dehydration in ethyl alcohol at ascending grades, clearing in zylol, blocking in paraffin wax,

sectioning by microtome at thickness of 4-6 μm, and mounting on glass slides. Then slides were deparaffinzed and stained with hematoxy line and eosin stains (Al-Forkan et al., 2016). Sections were examined for any pathological lesions in the histological structure of liver and kidney using light microscope (x100-400).

One-way ANOVA design was used for statistical analyses of all data by GLM procedures of SAS (2002). Completely randomized design was according the following statistical model:

$Y_{ij} = \mu + A_i + e_{ij}$.

Where: Y_{ij} = Observed values, μ = Overall mean, A_i = Effect of treatment (1…3) and e_{ij} = Random error.

However, conception and viability rates were statistically analyzed using Chi-Square test. Means with significant differences were set at a level of $P<0.05$ according to Duncan (1955).

RESULTS AND DISCUSSION

Effect of Spirulina platensis administration on:

Average of body weight and feed intake during treatment period:

Doe rabbits were significantly ($P<0.05$) heavier in G2 than in G1 and G3 at end of treatment at 20-22 wk of age , but change in body weight (BW), as absolute increase or relative to initial BW at 16-18 wk of age significantly ($P<0.05$) increased in G2 and G3 than in G1.This was associated with significant ($P<0.05$) increase in daily feed intake (DFI) during treatment period in G2 and G3 than in G1, being significantly ($P<0.05$) higher in G2 than in G3 (Table 1).

Similarly, dietary addition of Spirulina platensis increased growth performance and feed intake of growing rabbits (Gerencser et al., 2012; El-Desoky et al., 2013), broiler chicks (Kharde et al., 2012; Zeweil et al., 2016) and only feed intake of growing pigs (Nedeva et al., 2014) as compared to control. Also, El-Sabagh et al. (2014) found that dietary adding Spirulina platensis had beneficial effects on final BW, feed intake and feed conversion rate of fattening lambs as compared to controls.

This indicated higher impact of Spirulina platensis treatment at a level of 300 than 600 mg/doe on BW and DFI of doe rabbits. Increasing DFI of both treated groups (G2 and G3) may reflect effect of Spirulina platensis on increasing apatite of does in both treated groups as compared to control does. Improving the BW of treated doe rabbits may be reflecting high value concentrated nutritional compounds and phytopigments of Spirulina platensis (Borowitzka, 2013). It was reported that Spirulina platensis contained crud protein between 50 and 70% (on DM basis) with essential amino and fatty acids, photosynthetic pigments, vitamins, minerals, carotenoids, chlorophyll, pigments and essential polyunsaturated fatty acids (Mendes et al., 2003; Babadzhanov et al., 2004; Bermejo et al., 2008; Hoseini et al., 2013; Peiretti and Meineri, 2011; Farag et al., 2016).

Reproductive performance of doe and kid rabbits:

Reproductive measurements:

Reproductive measurements, in terms of conception rate, size and weight of litter at birth and weaning of does, as well as, viability rate and BW at birth and weaning of bunnies significantly ($P<0.05$)

increased in G2 and G3 than in G1, being the highest in G2. While, gestation period length and kindling rate were similar in G2 and G3 than in G1, but the difference was not significant (Table 2).

Similarly, several authors discovered positive impact of Spirulina platensis on reproductive parameters in lambs (El-Sabagh et al., 2014), poultry (Shanmugapriya et al., 2015) and mice (Pankaj, 2015). The positive effect of Spirulina platensis on reproductive measurements proved in the current study could be attributed to strong natural antioxidant (Abdel-Daim et al., 2015). In this respect, Spirulina platensis had synergetic effect of the chemical constituents, in terms total phenolic and flavonoid compounds (Zeweil et al., 2016). Also, Spirulina platensis has neuro-protective (Aziz et al., 2014), anti-tumor (Konickova et al., 2014), Immuno-modulatory (Sahan et al., 2015) and anti-inflammatory (Abdel-Daim et al., 2015) properties.

Reproductive hormones:

Concentrations of reproductive hormones, estradiol 17-β (E2) and progesterone (P4) were significantly ($P<0.05$) higher, while E2/P4 ratio was significantly ($P<0.05$) lower in blood plasma of does in G2 and G3 than in G1. However, only E2 concentration was significantly ($P<0.05$) higher in G2 than in G3 (Table 3). The observed improvement in E2 and P4 as reproductive hormones in blood plasma of treated doe rabbits may be attributed to direct effect of Spirulina platensis on secretion of these hormones from the follicles and CLs on the ovaries and/or indirect effect of Spirulina platensis on GnRH secretion from hypothalamus. In this respect, Spirulina platensis had a vital role on reproductive efficiency, which may be due to its contents of all essential amino acids (Farag et al., 2016), essential fatty acids (Mendes et al., 2003), photosynthetic pigments (Bermejo et al., 2008), vitamins (Hoseini et al., 2013), major and minor minerals (Babadzhanov et al., 2004) and essential polyunsaturated fatty acids (Peiretti and Meineri, 2011).

Hematological parameters:

Treatment of does with Spirulina platensis in G2 and G3 significantly ($P<0.05$) increased hemoglobin (Hb) concentration, count of red blood cells (RBCs) and hematocrit value (Ht), neutrophils and eosinophils, while significantly ($P<0.05$) decreased platelets count and lymphocytes, monocytes and acidophils percentages as compared to G1. On the other side, white blood cells (WBCs) count significantly decreased only in G2, while erthrocytic values of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were not affected significantly by treatment (Table 4).

It is of interest to note that increasing RBCs count in both treatment groups was associated with increasing Hb concentration and Ht value as affected by SP in G2 and G3 as compared to G1. Also, the observed reduction in WBCs count was in relation with reducing lymphocytes percentage and acidophils and marked increase in neutrophils (Table 4).

All hematological values obtained in this study on doe rabbits are within the normal ranges of rabbits (Olabanji et al., 2007; Togun et al., 2007). In accordance with the present results, dietary addition of Spirulina platensis enhanced

hematopoiesis (Hb concentration and RBCs count) in growing pig (Nedeva et al., 2014), broiler chicks (Zeweil et al., 2016), cow (Shimkus et al., 2008) and weaning lambs (Shimkiene et al., 2010).

It is well known that hematological characteristics are good indicators of animal physiological status (Khan and Zafar, 2005). In this way, Ht, Hb and MCH are major indices for evaluating circulatory erythrocytes (Chineke et al., 2006). Also, lymphocytes are considered the main type of WBCs and a good indicator of the immunity (Wieslaw et al., 2006). In rabbits, satisfactory nutritional status was indicated by Ht value (Church et al., 1984), while high dietary protein quality and animal free disease correlated with enhancing count of RBCs (Ahemen et al., 2013).

Improving most hematological parameters after Spirulina platensis treatment in this study might be related to the strong antioxidant effect of Spirulina platensis on hematopoietic cells, which appears to be particularly vulnerable in the presence of unchecked accumulation of reactive oxygen species, ROS (Kong et al., 2004). Also, the benefits of Spirulina platensis on hematological parameters may be due to the high content of folic acid and vitamin B_{12} in Spirulina platensis (Nedeva et al., 2014). Based on the present findings and previous results, doe rabbits treated with Spirulina platensis, in particular, at a level of 300 mg/ doe may improve health status.

Biochemical parameters and enzyme activity:

Treatment of does with Spirulina platensis in G2 and G3 significantly ($P<0.05$) increased total proteins, albumin, globulin and High density lipoproteins (HDL) concentrations, while it significantly ($P<0.05$) decreased total lipids, triglycerides, total cholesterol and Low density lipoprotein (LDL) concentrations, and activity of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Acid phosphatase (ACP) and Alkaline phosphatase (ALP) in blood plasma in G2 and G3 as compared to G1. However, glucose concentration was not affected by Spirulina platensis treatment (Table 5).

Similar effects were reported on concentration of total proteins and albumin in rabbit fed diet contained Spirulina platensis (Kovács et al., 2016), total proteins and globulin in pigs (Nedeva et al., 2014) and total proteins, albumin and globulin in broiler chicks (Zeweil et al., 2016). In human, concentration of total lipids, total cholesterol, triglycerides and LDL significantly ($P<0.05$) decreased with dietary supplementation of Spirulina platensis (Ramamoorthy and Premakumari, 1996), rats (Mazzola et al., 2015) and Holstein calves (Heidarpour et al., 2011) as compared to control. Moreover, Spirulina platensis supplementation to diet of fattening lambs decreased cholesterol and glucose compared with the control (El-Sabagh et al., 2014). The recorded increase in plasma total proteins, albumin and globulin concentrations may be related to high contents of protein, essential amino acids, vitamins, minerals, phospholipids and antioxidants in Spirulina platensis (Gershwin and Belay, 2008; Farag et al., 2016).

Activity of AST and ALT are indicators of hepatotoxicity and liver function (Azab et al., 2013). Regarding the effect of Spirulina platensis on enzyme activity, similar effects was reported on fattening lambs fed diet contained Spirulina platensis (El-Sabagh et al., 2014). In the present study, treatment with Spirulina platensis showed a significant decrease in AST and ALT indicating that Spirulina platensis may play a protective role against liver dysfunctions (Bhattacharyya and Mehta, 2012).

These results may indicate that Spirulina platensis had positive effect on protein metabolism, lipid profile and liver function of treated doe rabbits, consequently healthy status of doe treated rabbits.

Blood oxidative capacity:

Total antioxidant capacity (TAC), glutathione S-transferase (GST), superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxidase (GPx) increased significantly ($P<0.05$), while thiobarbituric acid-reactive substances (TBARS) concentration decreased significantly ($P<0.05$) in blood plasma of doe rabbits in G2 and G3 than in G1 (Table 6). In accordance with the present results, dietary supplementation of Spirulina platensis significantly increased GPx activity in rabbit (Kovács et al., 2016) and mice (Hwang et al., 2011), and TAC activity in chickens (Zeweil et al., 2016) when compared with control. Also, Spirulina platensis dietary supplementation significantly improved blood antioxidant status of fattening lambs (El-Sabagh et al., 2014), and activity of erythrocytic SOD and catalase with increasing content of reduced tripeptide GSH in chickens (Reddy et al., 2004).

These findings are good indicators of improving oxidative defense system (Celli, 2010) by reducing oxidative stress and consequently decreasing lipid peroxidation (Riss et al., 2007) of treated doe rabbits as compared to control ones. The SOD in Spirulina platensis acts indirectly by slowing down the rate of ROS (Belay, 2002).

In general, effect of Spirulina platensis, as a natural antioxidant, is related to many active ingredients, such as α-tocopherol, ß-carotene, phycocyanin and polysaccharides, which have strong activity of scavenging acting, individually or together, directly on superoxide radicals (Kurd and Samavati, 2015). In this respect, Gershwin and Belay (2008) found that the antioxidant capacity is about ≥20 times efficient in phycocyanin than in vitamin C.

Histological examination of liver and kidney:

The histological examination of liver of doe rabbits in all groups revealed that liver showed normal and intact architecture in doe rabbits of all groups. The liver was consisted of normal hepatic lobules with central hepatic vein, being with more dilation in G2 and G3 than in G1. All hepatic lobules were without limiting connective tissues at their lateral borders or between the hepatic lobules. Also, normal hepatocyte cords were radiated around the central hepatic vein within each hepatic lobule, being more arranged in G2 than in G1 and G3. Moreover, wall of central hepatic vein was thicker in G3 than in G1 and G2, but blood sinusoids were normal in all groups (Figure 1). These observations may

indicate normal liver function in doe rabbits in treated groups, with some beneficial effect on arrangement of hepatocyte cords in G3. The observed improvement of histological structure of liver, particularly in G2 is in harmony with marked reduction in lipid peroxidation induction via free radical scavenging activity in goat liver homogenates (Ray et al., 2007). Also, Spirulina platensis decreased oxidative stress and apoptosis in liver of animals fed diets contaminated with aflatoxins (Hassan et al., 2012). Furthermore, Garcia-Martinez et al. (2007) mentioned that Spirulina platensis had natural antioxidant components, having strong activity of scavenging acting to give its hepato-protective potential.

The histological examination of kidney of doe rabbits in all groups revealed that kidney in all groups showed normal architecture of renal cortex and medulla. Several Nephrone units and numerous renal tubules were found renal cortes of doe rabbits in all groups, but Nephron units were denser in G2, with moderate density in G3 and lowest density in G1 (Figure 2). Magnification of the previous sections in kidney showed normal nephron units in the renal cortex with intact glumerulosa and normal Bowman's capsul as well as circular proximal renal tubules were observed in all groups, but Nephron units were larger in G2 than in G3 and G1 (Figure 3). As proved in liver, the present findings may indicate normal kidney function in doe rabbits in treated groups. In consistent with obtained observations on kidney histogenesis of treated does, Khan et al. (2006) reported that Spirulina had a protective effect against nephron-toxicity in rats through reducing kidney MDA and pathological changes in kidney. Also, Spirulina platensis decreased lipid peroxidation, increased SOD, GSH and GPX levels and improved histological picture of kidney of rats with renal dysfunction (Karadeniz et al ., 2008). Moreover, drinking water supplemented with Spirulina platensis had protective properties against nephrotoxicity induced by chromium in term of decreasing of histological alterations in rats (Elshazly et al., 2015).

CONCLUSION

The potential application of orally administrated Spirulina platensis (300 mg/doe) as natural antioxidants to protect doe rabbits against free radicals cellular damage under oxidative stress, was proved to improve reproductive performance, blood constituents, antioxidative status without adverse effects on liver and kidney functions. Therefore, Spirulina has several advantages due to their applications as production of safe and healthy food and animal feed for therapeutic practices.

Table (1): Effect of Spirulina platensis administration on body weight and daily feed intake of doe rabbits during treatment period from 16-18 to 20-22 wk of age.

Parameter (16-18 to 20-22 wk of age).	Control (G1)	Spirulina platensis level		±SEM
		G2 (300 mg/doe)	G3 (600 mg/doe)	
Initial body weight (g/doe)	2630.3	2624.3	2621.1	3.345
Final body weight (g/doe)	2885.8^{b}	2901.0^{a}	2891.7^{b}	3.216
Chang in body weight (g/doe)	255.5^{b}	276.7^{a}	270.7^{a}	2.719
Chang in body weight (%)	9.71	10.54	10.33	-
Daily feed intake (g/doe)	153.13^{c}	161.27^{a}	157.67^{b}	0.588

Means denoted within the same row with different superscripts are significantly different at P<0.05.

Table (2): Effect of Spirulina platensis administration on reproductive performance of doe rabbits and performance of their bunnies.

Parameters	Control (G1)	Spirulina platensis level		±SEM
		G2 (300 mg/doe)	G3 (600 mg/doe)	
Number of does	15	15	15	-
Body weight at kindling (g/doe)	3000.57^{c}	3027.47^{a}	3012.80^{b}	3.467
Conception rate (%)	64.29^{b}	93.33^{a}	86.67^{a}	-
Kindling rate (%)	100	100	100	-
Gestation period length (day)	31.11	31.07	31.23	0.296
Total litter size at birth/doe (n)	6.12^{c}	8.29^{a}	7.46^{b}	0.301
Live litter size at birth/ doe (n)	5.11^{c}	8.00^{a}	6.92^{b}	0.245
Viability rate at birth (%)	83.49^{b}	96.50^{a}	92.76^{a}	-
Litter size at weaning/doe	4.44^{c}	7.93^{a}	6.77^{b}	0.228
Viability rate at weaning (%)	86.89^{b}	99.13^{a}	97.83 ^{a}	-
Average bunny weight at birth (g)	54.89^{b}	58.36^{a}	58.15^{a}	0.895
Average bunny weight at weaning (g)	419.78^{c}	450.21^{a}	439.77^{b}	1.587
Litter weight at birth (g)	280.48^{b}	466.88^{a}	402.40^{a}	2.625
Litter weight at weaning (g)	1863.82^{b}	3570.16^{a}	2977.24^{a}	8.216

Means denoted within the same row with different superscripts are significantly different at P<0.05.

Table (3): Effect of Spirulina platensis administration on concentration of reproductive hormones in blood plasma of doe rabbits on day 15 post-mating.

Parameter	Control (G1)	Spirulina platensis level		±SEM
		G2 (300 mg/doe)	G3 (600 mg/doe)	
E2 (ng/ml)	0.148^{c}	0.199^{a}	0.192^{b}	0.0012
P4 (ng/ml)	0.346^{b}	0.553^{a}	0.529^{a}	0.0079
E2/P4 ratio	0.428^{a}	0.360^{b}	0.364^{b}	0.0067

Means denoted within the same row with different superscripts are significantly different at $P<0.05$.

Table (4): Effect of Spirulina platensis administration on hematological parameters of doe rabbits at the end of treatment period. (All values are within normal range)*

Parameter	Control (G1)	Spirulina platensis level		±SEM
		G2 (300 mg/doe)	G3 (600 mg/doe)	
haematological parameters				
Hb (mg/dl)	12.08^{c}	14.46^{a}	13.97^{b}	0.053
RBCs (x 10^6/mm^3)	6.07^{b}	6.81^{a}	6.59^{a}	0.033
WBCs (x 10^3/mm^3)	8.03^{a}	7.74^{b}	7.84^{ab}	0.0638
Platelets (x 10^3/mm^3)	286.40^{a}	180.40^{c}	233.80^{b}	3.722
Ht (%)	38.20^{c}	46.60^{a}	41.600^{b}	0.868
MCV (μ^3)	62.20	63.00	62.60	0.856
MCH (pg)	22.40	22.80	22.80	0.6164
MCHC (g/dl)	32.40	33.00	32.60	0.841
Leucocyte fraction (%):				
Lymphocytes	50.40^{a}	39.50^{c}	42.40^{b}	0.624
Monocytes	5.11^{a}	3.97^{b}	4.14^{b}	0.0798
Neutrophils	41.04^{c}	53.29^{a}	50.20^{b}	0.663
Acidophils	2.16^{a}	1.11^{b}	1.22^{b}	0.053
Eosinophils	1.29^{b}	2.13^{a}	2.04^{a}	0.099

Means denoted within the same row with different superscripts are significantly different at $P<0.05$. * Normal range according to Steven et al. (1974).

Table (5): Effect of Spirulina platensis administration on some biochemical concentrations and enzyme activities in blood plasma of doe rabbits at the end of treatment period.

Parameter	Control (G1)	Spirulina platensis level		±SEM
		G2 (300 mg/doe)	G3 (600 mg/doe)	
Biochemical concentration:				
Total proteins (g/dl)	6.41^{c}	7.50^{a}	7.18^{b}	0.0296
Albumin (g/dl)	3.39 ^{c}	3.51^{a}	3.44^{b}	0.0145
Globulin (g/dl)	3.02^{c}	3.99^{a}	3.74^{b}	0.035
Glucose (mg/dl)	92.30	92.20	92.00	0.748
Total lipids (mg/dl)	318.00^{a}	200.80^{c}	222.20^{b}	4.725
Total cholesterol (mg/dl)	96.20^{a}	81.20^{b}	85.20^{b}	1.332
Triglycerides (mg/dl)	88.40^{a}	70.20^{c}	74.40^{b}	1.349
High density lipoproteins (mg/dl)	54.20^{c}	63.20^{a}	60.20^{b}	0.898
Low density lipoprotein (mg/dl)	43.80^{a}	30.60^{b}	33.61^{b}	1.058
Enzyme activity (IU/l):				
Aspartate aminotransferase	47.80^{a}	34.00^{c}	40.60^{b}	0.876
Alanine aminotransferase	32.20^{a}	21.00^{c}	24.80^{b}	1.029
Acid phosphatase	28.40^{a}	21.60^{b}	25.20^{a}	1.058
Alkaline phosphatase	49.80^{a}	43.00^{b}	44.00^{b}	0.762

Means denoted within the same row with different superscripts are significantly different at P<0.05.

Table (6): Effect of Spirulina platensis administration on oxidative capacity in blood plasma of doe rabbits at the end of treatment period.

Parameter	Control (G1)	Spirulina platensis level		±SEM
		G2 (300 mg/doe)	G3 (600 mg/doe)	
TAC (mmol/l)	0.27^{c}	0.38^{a}	0.32^{b}	0.018
GST (IU)	1.04^{c}	1.35^{a}	1.28^{b}	0.0947
SOD (IU)	6.22^{c}	7.48^{a}	7.02^{b}	0.094
GSH (mg/dl)	15.28^{c}	18.40^{a}	17.24^{b}	0.363
GPx(mg/l)	3.58^{c}	6.17^{a}	5.92^{b}	0.065
TBARS (nmol/ml)	1.10^{a}	0.93^{b}	0.95^{b}	0.021

Means denoted within the same row with different superscripts are significantly different at P<0.05.

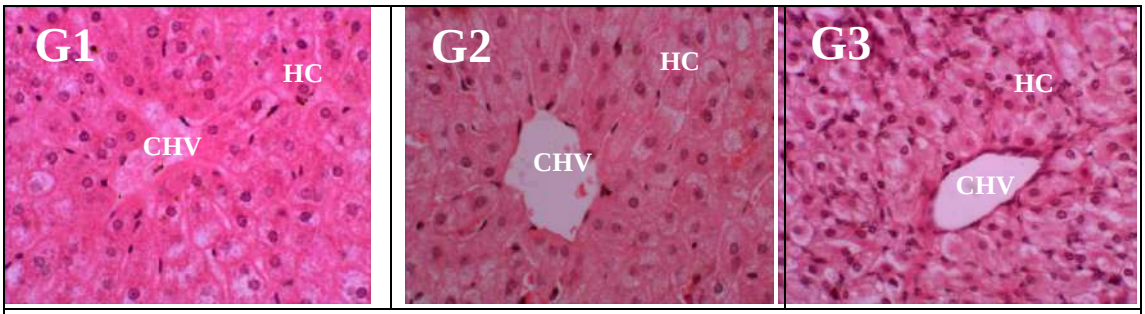

Fig. (1): Section in liver showing normal and intact architecture of liver doe rabbits in G1, G2 and G3. The liver is consisted of normal hepatic lobules with central hepatic vein (CHV) and hepatocytes (HC) arranged in radiated cords around CHV. CHV was more dilation in G2 and G3 than in G1. No connective tissues in limited borders between hepatic lobules were seen and normal hepatocyte cords were radiated around the CHV, being more arranged in G2 than in G1 and G3. Wall of CHV is thicker in G3 than in G1 and G2. Blood sinusoids are normal in all groups. (H & E stain, x 100)

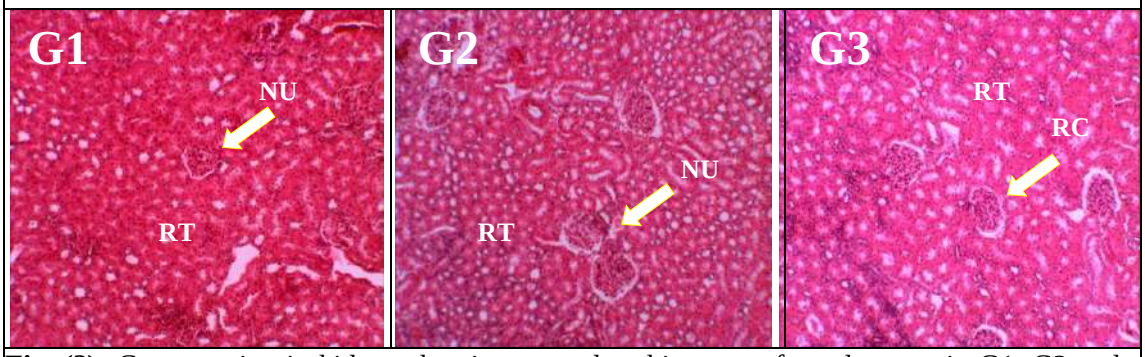

Fig. (2): Cross-section in kidney showing normal architecture of renal cortex in G1, G2 and G3. Several Nephrone units (NUs) and numerous renal tubules (RT) are seen in all groups, but NU are denser in G2,with moderate density in G3 and lowest density in G1.(H & E stain,x 40)

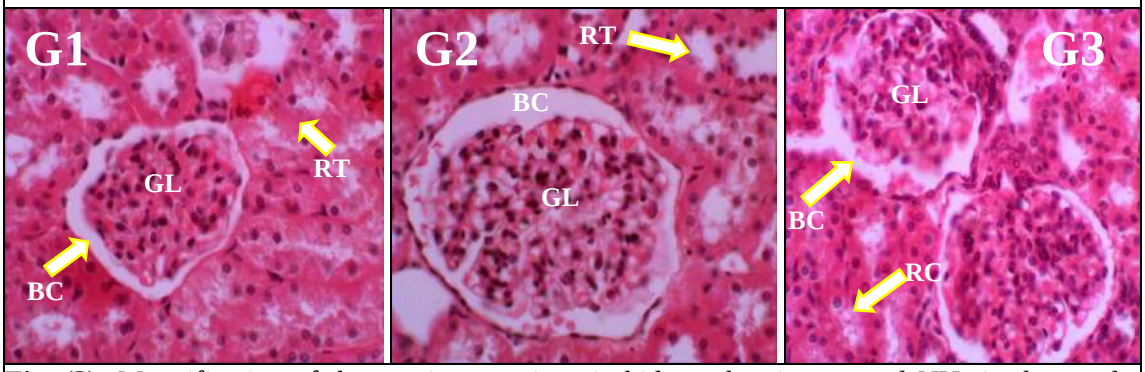

Fig. (3): Magnification of the previous sections in kidney showing normal NUs in the renal cortex with intact glumerulosa (GL) and normal Bowman's capsul (BC), as well as, circular proximal renal tubules (RT) are seen in all groups, but NUs are larger in G2 than in G3 and G1. (H & E stain, x 100)

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الملخص العربى

الأداء التناسلى، حالة الأكسدة والمواد التمثيلية فى دم أمهات الأرانب المعاملة بطحلب الإسبيرولينا

ابراهيم طلعت الرطل
قسم إنتاج الدواجن – كلية الزراعة – جامعة دمياط – مصر.

أجريت هذه التجربة بمزرعة أرانب خاصة تقع بقرية طنيخ، مدينة نبروه، محافظة الدقهلية، مصر، بينما أجريت التجارب المعملية بمعمل الفسيولوجى والبيوتكنولوجى، قسم الإنتاج الحيوانى، كلية الزراعة، جامعة المنصورة، مصر وذلك فى الفترة من مارس حتى يونيه 2017.

تهدف هذه الدراسة الى تقييم تأثير تجريع أمهات الأرانب بمستويات مختلفة من طحلب الإسبيرولينا على الأداء التناسلى، خصائص الدم الهيماتولوجية، البيوكيميائية، نشاط مضادات الأكسدة والتركيب الهستولوجى للكبد والكلية.

إستخدمت فى هذه التجربة عدد 45 من أمهات الأرانب الأبرى ذات عمر من 16-18 أسبوع ولم يسبق لها الولادة، قسمت الى 3 مجموعات (15/مجموعة). جرعت أمهات المجموعة الأولى (كنترول)، الثانية والثالثة يوميا بـ 3مل ماء مقطر يحتوى على صفر،300 و600 ملجرام/أم من طحلب الإسبيرولينا لمدة 4 أسابيع (مدة المعاملة) على التوالى. تم تلقيح الأمهات طبيعيا فى نهاية فترة المعاملة بـ15 ذكر أبرى ناضج جنسيا (5 ذكر/مجموعة) غير معامل. تم تسجيل وزن الأمهات فى بداية ونهاية المعاملة (16-18 و20-22 أسبوع على التوالى) وعند الولادة (22-26 أسبوع) ووزن الخلفات عند الميلاد والفطام. تم حساب معدل استهلاك الغذاء للأمهات يوميا خلال فترة المعاملة. تم حساب معدلات الحمل، الولادة وحجم البطن (الكلى-الحى) للأمهات وكذلك معدل الحيوية للخلفات عند الميلاد والفطام. تم تقديرتركيز هرمونى الاستراديول والبروجسترون فى بلازما دم الأمهات فى اليوم الـ15 بعد التلقيح. قدرت خصائص الدم الهيماتولوجية، البيوكيميائية ونشاط مضادات الأكسدة فى بلازما الدم للأمهات فى نهاية المعاملة. تم فحص تركيب أنسجة الكبد والكلية للأمهات هستولوجيا.

وقد أظهرت النتائج: زيادة معنوية ($P<0.05$) فى وزن الأمهات فى نهاية المعاملة فى المجموعة الثانية مقارنة بالمجموعة الثالثة والكنترول مع زيادة معنوية ($P<0.05$) للتغيرفى وزن الجسم كقيمة مطلقة أو كنسبة الى وزن الجسم فى بداية التجربة وكذلك معدل استهلاك العلف اليومى للأمهات فى المجموعة الثانية والثالثة مقارنة بالكنترول. وجد زيادة معنوية ($P<0.05$) فى معدل الإخصاب، حجم البطن، معدل الحيوية ووزن الجسم للخلفات عند الميلاد والفطام مع تشابة معدل الولادات فى كل المجموعات. لوحظ زيادة معنوية ($P<0.05$) فى تركيز هرمونى الاستراديول والبروجسترون فى بلازما الدم، بينما انخفض معدل الاستراديول/ البروجسترون معنويا ($P<0.05$) فى المجموعة الثانية والثالثة مقارنة بالكنترول. كان تركيز البروجسترون اعلى معنويا ($P<0.05$) فى المجموعة الثانية عن الثالثة. لوحظ زيادة معنوية ($P<0.05$) فى تركيز الهيموجلوبين، عدد خلايا الدم الحمراء وقيمة الهيماتوكريت والنسبة المئوية للخلايا المتعادلة والحامضية فى المجموعة الثانية والثالثة، مع انخفاض معنوى ($P<0.05$) فى عدد الصفائح الدموية والنسبة المئوية للخلايا الليمفاوية والأحادية والقاعدية فى المجموعة الثانية والثالثة مقارنة بالكنترول. انخفض عدد خلايا الدم البيضاء معنويا ($P<0.05$) فى المجموعة الثانية فقط. وجد زيادة معنوية ($P<0.05$) فى تركيزات البروتينات الكلية والالبيومين والجلوبيولين والليبوبروتينات مرتفعة الكثافة، مع انخفاض معنوى ($P<0.05$) فى تركيزات الدهون الكلية، الجلسرول، الكولستيرول الكلى والليبوبروتينات منخفضة الكثافة فى بلازما الدم للمجموعة الثانية والثالثة مقارنة بالكنترول. أدت المعاملة الى زيادة معنوية ($P<0.05$) فى محتوى الإنزيمات المضادة للأكسدة مع انخفاض معنوى ($P<0.05$) فى تركيز الشوارد الحرة فى المجموعة الثانية والثالثة مقارنة بالكنترول. لم يلاحظ وجود أى تغيرات غير مرغوبة في التركيب النسيجي للكبد والكلى.

نستخلص من هذه الدراسة: تجريع أمهات الأرانب يوميا بطحلب الإسبيرولينا (300ملجرام/أم) لمدة 4 أسابيع قبل التلقيح كمضاد أكسدة طبيعى له تأثير ايجابى ملحوظ على الأداء التناسلى، مكونات الدم وحالة مضادات الأكسدة مع وظائف طبيعية لكل من الكبد والكلية.