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The Effects of Dietary Supplementation of *Chlorella vulgaris* Alga and *Lactobacillus reuteri* Bacteria in Female Rabbit Puberty Subjected to Heat Stress

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Abstract

Chlorella vulgaris Alga (*C.vulgaris*) is a highly nutritious microalgae, Also *Lactobacillus reuteri* Bacteria (*L.reuteri*) produce a high amount of phytase enzyme that liberates necessary minerals for health. The present work aimed to study the enhancement effects of *C.vulgaris* and *L.reuteri* on the puberty of young rabbit-doe exposed to heat stress (HS). 28 young New Zealand rabbit-does were used and divided into four groups (n=7). (G1) was used as a negative control group. (G2) exposed to heat stress. (G3) & (G4) dietary supplemented with *C.vulgaris* (60mg) and *L.reuteri* (count producing 250 phytase units)/rabbit/day for 4 weeks, respectively. The live weight, feed intake, daily gain, and food conversion ratio were recorded. Plasma chemistry was estimated for the liver and kidney function parameters as Albumin, Creatinine, Alanine-Transaminase(ALT), Aspartate-aminotransferase(AST), and the antioxidant markers Catalase and Super Oxide Dismutase(SOD), Also the oxidative stress factor Malondialdehyde(MDA), in addition to the reproductive hormones progesterone(P4), follicle-stimulating-Hormone(FSH), Luteinizing-Hormone(LH), and cytokines Interlukine-1(IL-1), Tumor-Necrosis-Factor α(TNFα), Gamma-Interferon(INFϒ), and Transforming-Growth-Factor-Beta-2($TGFB_2$). Tissue samples from ovary, liver, small intestine, and spleen were examined histopathologically. The *C.vulgaris* and *L.reuteri*-supplemented groups showed significant up-regulation for the antioxidant markers and reproductive hormones. They also, marked down-regulation for the liver and kidney function enzymes, and oxidative stress factor (MDA). Moreover, a marked increase in growing and mature follicles was found. In conclusion, *C.vulgaris* and *L.reuteri* enhanced the rabbit puberty and ameliorated the adverse heat stress effects by promoting the growth rate, oxidative status, and ovarian follicular activity.

Keywords: *algae – bacteria - puberty-growth-ovary*.

Introduction

Rabbits are susceptible to heat stress (HS) due to the absence of sweat glands. The thermo-neutral zone temperature in rabbits is around 18–21°C. HS negatively affects feed intake; feed utilization; and water metabolism. It also affects reproductive traits, hormonal secretion, and the utilization of protein, energy, and minerals [1-2]. Puberty is associated with the production of certain levels of sex hormones such as progesterone, estrogen, and testosterone which were negatively affected by heat stress, and hepatic dysfunction in the biosynthesis of cholesterol required for sexual hormone production [3].

Chlorella vulgaris (*C.vulgaris*) is a highly nutritious unicellular freshwater microalgae that is rich in numerous valuable substances such as proteins, carbohydrates, vitamins, fatty acids, and trace elements like zinc, copper, and magnesium which are crucial for the function of antioxidant metalloenzymes and necessary for reproductive patency [4]. Moreover, *C.vulgaris* is rich in polyunsaturated fatty acids (PUFAs) which have been found to potentiate fertility [5-6]. *C.vulgaris* was reported as a natural growth promoter, immune booster, and tissue rebuilder [7].

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Rabbits possess insufficient inherent phytase activity. The phytate-rich grain worsens the mineral status needed for health. Phytate is a polyanionic molecule that has the potential to chalet the positively charged nutrient and hinder the utilization of protein/amino acids, calcium, and trace elements like zinc, copper, iron, iodine, magnesium, and selenium. These elements are essential for immune function and reproductive performance [8].

An earlier study showed that *Lactobacillus reuteri* (*L.reuteri*) *(L-M15) and Lactobacillus salivarius (L-ID15)* had high phytase production and phytate degrading activity when used as a starter in the whole wheat bread-making process [9] when *Lactobacillus reuteri* (*L.reuteri*) fed to birds showed high phytase activity expressed as liberation of the bound phosphorus leaving the least amount of phytin-phosphorus in birds' ingesta [10].

The big goal for this study was to find the effect of supplementation of the microalgae *C.vulgaris* and *L.reuteri*-producing microbial phytase to enhance puberty in young rabbit-doe suffering from heat stress.

Material and Methods

Chemical analysis of the nutrients of microalgae

Experimental samples were analyzed to estimate dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), ether extract (EE), and ash contents. Carbohydrates (nitrogen-free extract; NFE) were also estimated by difference according to the standard methods of the Association of Official Analytical Chemists [11].

Phytase Assay

Phytase activity was determined according to the method of Awad *et al.* [10] by measuring the amount of liberated inorganic phosphate one unit of the phytase activity was expressed as the amount of enzyme required to liberate 1ìmol of phosphate per minute from sodium phytate.

Determination of LAB growth

L. reuteri concentration was determined by measuring the optical density (OD) of its culture broth at the wavelength of 600 nm, then comparing it to standard curves previously obtained by relating colony forming units (CFU) per mL from plate counts and OD measurements [12].

Preparation of bacterial suspension

Lactobacillus reuteri was activated in MRS media. 0.1ml of bacterial suspension had $1.5x10^5$ CFU/ml that was presumably producing 100 phytase unit (FTU). The enzyme activity was determined by measuring the amount of liberated inorganic phosphate [10].

Determination of the Total antioxidant activity of Chlorella vulgaris

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay was carried out according to the method of Beltagi *et al.* [13].

Antimicrobial activity

Antimicrobial activity of Chlorella vulgaris

The studied organisms were Gram-positive bacteria like *Staphylococcus aureus*; NRRL B-313, Gram-negative bacteria *Escherichia coli;* NRRL B-210, and *Pseudomonas aeruginosa* NRRL B23 27853 and *L. reuteri*. These microorganisms were obtained from the National Research Center/Department of Chemistry of Natural and Microbial product/Cairo/Egypt and were grown and maintained in nutrient agar media (Difco 0001) [14].

Antimicrobial activity of lactobacillus reuteri

Bacteria were studied against the same types of pathogens [14].

Biological study

Housing and formulation of the basal diet

All experimental rabbits were kept under the same managerial, hygienic, and environmental conditions (before the application of the heat-stress environmental condition). Growing rabbits were housed in galvanized wire batteries provided with feeders and automatic stainless-steel nipples to supply each cage with water all the time. All batteries were in an open rabbitry and exposed to natural environmental temperature and photoperiod and ventilated by windows and fans. Ceiling electric fans were also used when needed. Growing rabbits were fed on an experimental diet (Table 1) to cover their requirements according to recommendations.

Animal Management and Ethical Approval

The authors confirm that the ethical policies were proven and the appropriate ethical review committee approval has been received for the project number (13050419-NRC, Egypt). The authors followed EU standards for the protection of the rabbits used for scientific purposes. The study was conducted at the rabbitry of the Faculty of Agriculture/Al-Azhar University/Cairo/Egypt. The microalgae were provided by the Algae Biotechnology Unit/National Research Center (NRC)/Dokki/ Egypt. Nutritional analysis (protein, carbohydrates, Lipids, minerals, and trace elements) was done in the atomic absorption unit in NRC.

Experimental design

Twenty-eight New Zealand female rabbits of body weight 1500.00±0.091gm were used in this study. Rabbits were randomly divided into four equal groups (n=7). (G1) fed commercial diet (CD) not

exposed to heat stress, and kept in an air-conditioned room. (G2) fed CD and exposed to heat stress. (G3) fed CD and given by stomach tube suspension of *C.vulgaris* 60 mg/rabbit/day/4 weeks. (G4) fed CD and given *L. reuteri* count producing phytase unite (FTU) 250 unit/rabbit/day/4 weeks.

Heat stress room environmental condition

The heat stress room was equipped with thermometers and humidity-measuring equipment, the minimum and maximum average temperatures were measured and recorded in Table (2). The Average temperature-humidity index (THI) was predestined regularly based on [15] as follows: THI = Tdb – (0.31 - 0.31 RH) (Tdb - 14.4). Where Tdb presents the dry bulb temperature in Celsius; RH presents the relative humidity percentage. The corresponding stress levels for the following THI values <27.8, 27.8 to <28.9, 28.9 to <30.0, and ≥30 were identified as no stress, moderate, severe, and very severe heat stress, respectively. As it is clear from the table that; animals suffer from severe to very severe heat stress.

Growth performance

The live weight and feed intake were recorded weekly. The average daily feed intake, average daily body gain, and feed conversion ratio were calculated for the estimation of the production performance.

Blood parameters

Individual blood samples were taken from the marginal ear vein and collected in 5 ml heparinized test tubes and centrifuged at 3000 r.p.m for 20 minutes, then plasma was transferred and stored at -20º C till analysis of plasma chemistry parameters as; liver and kidney function markers as; Albumin, Creatinine, Alanine Transaminase (ALT), Aspartate aminotransferase (AST), and the oxidative stress marker as; Malondialdehyde (MDA), Also the antioxidant markers; Catalase and superoxide dismutase (SOD), in addition to the reproductive hormones as; Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), and progesterone (P4), and cytokines as; Interlukine-1 (IL-1), Tumor Necrosis Factor α (TNFα), Gamma Interferon (INFϒ), and the proliferative marker Transforming Growth Factor Beta-2 ($TGFB_2$). These parameters were measured by using an ELISA commercial kit (SUNLONG, China). The sensitivity of the assay was 0.05ng/ml for rabbit P4, 0.1ng for FSH, 0.01 ng/ml for LH, 0.5 pg/ml for INFϒ, 0.1pg/ml for TNFα, 0.6 pg/ml for $TGFB_2$, and 0.7 pg/ml for IL-1. The intra and inter-assay precisions for all the ELISA kits were <10% and <12%. The total protein, albumin, cholesterol, ALT, AST, creatinine, MDA, SOD, and catalase were analyzed using colorimetric diagnostic kits (Biodiagnostic, Egypt).

Histopathological Examination

A histopathological examination was performed on all rabbits at the end of the experiment. Tissue specimens from the ovary, intestine, liver, and spleen were picked up for the detection of histopathological changes. Tissue specimens were fixed in 10% neutral buffered formalin (NBF). Routine tissue processing was done as dehydration, embedding in paraffin wax, and sectioning at 3–5 µm. The prepared tissue slides were stained with H&E for histopathological examination [16].

Statistical analysis

Statistical analysis for the data was performed by one-way analysis of variance (ANOVA) using SPSS [17] software (Ver. 20.0 for Windows, SPSS, Inc., Chicago, IL). The replicate was used as an experimental unit (n=5). Duncan's multiple range test was used to test the significant differences among the mean values [18]. The pooled standard error of the mean (SEM) was listed for all analyses. The significant level for differences was set as $P < 0.05$.

Results & Discussion

Nutritive value of Chlorella vulgaris

The nutritive value of *C.vulgaris* is represented in Table (3) which reveals the enrichment of *Chlorella* alga with protein. Moreover; Table (4) showed that; *Chlorella* is a rich source of calcium, magnesium, phosphorus, and traces like zinc, copper, and selenium.

These nutritive values of *C.vulgaris* alga were clarified to us the consideration of the use of microalgae as a source of protein for animals and humans [19]. Moreover, Copper is essential for zinc and iron activity as metalloenzymes which regulate plenty of biochemical processes and act as antioxidants to prevent protein degeneration and DNA fragmentation [20]. *Chlorella* is rich in zinc which has crucial roles in the development of immunological and reproduction gene expression [21].

Determination of DPPH free-radical scavenging activity:

The present results revealed that; *Chlorella* has high antioxidant power expressed as 81% inhibition of DPPH as compared to ascorbic acid (94%) which is considered as a control.

These findings were confirmed by Mtaki *et al.* [22] who reported the highest antioxidant contents and their free radical scavenging ability in the *C.vulgaris* alga extract decreases the risk of degenerative disease conditions such as cancer,

diabetes, aging, inflammation, stroke, and neurodegenerative disease in living organisms.

Antimicrobial activity

The existing results in Table (5) revealed that; both *C. vulgaris* and *L. reuteri* have favorable antimicrobial activity against pathogenic bacteria as compared to commercial antibiotics.

It is well known that Lactic acid bacteria (LAB) produce antimicrobial factors and bacteriocins (bactericidal proteins). So, probiotics may be considered for the treatment and prevention of a variety of infectious diseases caused by oral, enteric, and urogenital pathogens [23-24]. Reuterin is a primary antimicrobial compound produced by *L. reuteri* bacteria during glycerol fermentation. *L. reuteri* is highly resistant to antimicrobial factors secreted by different strains belonging to the same species [25-26].

C. vulgaris alga is a rich source of phenolic compounds that have antioxidants and antimicrobial effects [27]. At the same time, *C.vulgaris* contains cyclic peptides, γ-linolenic acid which is a fatty acid that acts as an antimicrobial factor to inhibit the growth of both gram-positive and negative bacteria [28].

Growth performance

As shown in Fig. (1) Heat stress depressed feed intake and body weight gain to a great extent and consequently, the FCR and FER were in the worst case. Administration of *C.vulgaris* and *L.reuteri* greatly improved the condition. The body weight gain and FER showed significantly higher values than the control groups respectively.

A previous study stated that; the inclusion of *C.vulgaris* in animal diets caused increased concentration of some digestive bacterial species [29] resulting in improvement in feed utilization [30]. Moreover, it contains β-glucan, which plays a role in scavenging free radicals [31]. Other study recorded better feed utilization efficiency, feed intake, and better feed conversion ratio in young rabbits supplemented with *Chlorella* extract. Also, there was a significant difference in sex hormones of kits kindled by rabbits supplemented with *Chlorella* [32].

Concerning *L. reuteri*; a previous study reported that the bacteria produce a broad-spectrum antibiotic called reuterin [33] which enhances body weight gain and FER. At the same time, *L.reuteri* produces a phytase enzyme that acts on the bound form of phytate to liberate the hampered positively charged ions (Calcium, magnesium, zinc, copper, iron, and potassium) [34] and improve the amino acids utilization in the ileum after the breakdown of the phytin–protein complex [35]. Phytase improves the phosphorus digestibility by 14.7% and corrects the Calcium/phosphorus ratio which is essential for reproductive health [36].

Blood biochemical parameters

The current study showed the chemistry of blood plasma which is illustrated in Table (6). The rabbit-doe that was kept under heat stress (G2) showed significantly higher values for creatinine, ALT, AST, MDA, and INFγ compared to other groups ($P<0.01$) and low levels of catalase, SOD, LH, and $TGF\beta_2$.

These results could be explained as; the group of rabbits supplemented with *C.vulgaris* and *L.reuteri* and kept under heat stress (G3 and G4) showed down-regulation of liver enzymes, MDA and IL-1 and elevated the level of catalase, SOD, FSH, LH, TGF, and INF in comparison with heat stress rabbits (G2).

The changes in kidney and liver enzymes may be due to heat stress which can cause cellular damage and inflammation in rabbits [37]. this was confirmed in the current study by the detection of pathological changes in the liver of G2 subjected to heat stress and tissue recovery in G3 and G4 accompanied by higher antioxidant markers where some of the active constituents of *C.vulgaris* such as flavonoids, β-carotene, and phycocyanin have been reported to have noticeable antioxidant property and provoke free radical scavenging enzyme [38-39]. Also, *L.reuteri* lowered MDA by controlling pathogenic organisms and immunological markers [40].

Histopathological findings

Ovary

As illustrated in Fig. (2), the ovarian tissue of the control rabbits (Fig. 2A) showed complete normality for the ovarian tissue architecture, where it contained moderate numbers of the different follicular stages as primordial and mature follicles. The pronounced changes in (Fig. 2B) in the heat stress-exposed immature rabbits-doe (G2) were poor ovarian follicular activity which appeared as, the presence of few numbers of primordial follicles and at the same time, several degenerated and necrosed mature follicles were found.

Several authors supported and returned these changes to; severe follicular degeneration, oocyte lysis, and granulosa cell apoptosis in the heat stress-exposed rabbit doe and referred to these changes due to the elevation of the oxidative stress factors [41-43].

In the *C.vulgaris* algae-treated immature rabbit-doe (G3) the obvious findings in (Fig. 2C) indicated the safeness of this alga to the examined tissues and also protected the ovarian tissue from the heat stress effects where it exhibited a moderate increase of the ovarian follicular activity expressed as an increased number of primordial follicles beside a moderate number of mature follicles in comparison with the heat stress-exposed rabbits.

Moreover, the *L.reuteri*-treated immature rabbit doe exhibited favorable changes as compared to G2 where a small number of primordial follicles and a moderate number of mature follicles were seen (Fig 2D).

These results may be referred to the antioxidant effect of both alga and bacteria. As shown in Table (6) where both *C. vulgaris* and *L. reuteri* lowered the values of MDA and elevated others of SOD and catalase if compared to G2 that was subjected to heat stress.

Earlier studies reported and clarified a tight relationship between serum oxidative stress markers and the reproductive index of rabbit-doe [20], where both G3 and G4 in the present study showed up-regulation of FSH, LH, and Progesterone indicating better ovarian function. *L. reuteri* was reported among other lactic acid bacteria to improve steroidogenesis, gametogenesis, and fertility by increasing reproductive hormones [40].

Small intestine

As displayed in Fig (3) the intestinal tissue of the control rabbits (G1) displayed normal intestinal villi with intact columnar epithelium and normal tissue cores (Fig. 3A). The heat stress-exposed rabbits (G2) exhibited moderate degeneration and necrosis in the villus tissue cores accompanied by vacuolar degeneration in some of their lining epithelium (Fig. 3B).

These pathological changes could be attributed to heat stress which leads to metabolic and functional changes in various cells and tissues. It increases the production of transition metal ions, which can induce electron donations to oxygen-constituting superoxide or H_2O_2, which is later reduced to reactive oxygen species (ROS) leading to oxidative stress [40].

The *C. vulgaris*-treated rabbits illustrated normal intestinal villi with intact columnar epithelium and normal tissue cores (Fig. 3C).

These intestinal tissue findings support that; the antioxidant factors in *C. vulgaris* are responsible for improving the histological picture of the intestinal villi and keeping them with intact columnar epithelium and normal tissue cores

On the other side; the *L. reuteri*-treated rabbits exhibited intestinal tissue excitation which was detected as a severe thickening in the intestinal villi due to severe hyperplasia in their lining epithelium (Fig. 3D).

The proliferative effects of the *L. reuteri* bacteria on the intestinal lining epithelium could be explained as; *L. reuteri* in the small intestine secrete a specific metabolite indole-3-aldehyde which stimulates the lymphocytes in the lamina propria to release interleukin-22 that in turn accelerate the proliferation of intestinal epithelia, and so ameliorating damaged intestinal mucosa [44].

These findings may explain the ability of both algae and bacteria to improve body weight gain and FER in G3 and G4 as they improve the intestinal absorption of nutrients.

Liver

As detected in Fig. (4) The hepatic tissue of the control rabbits appeared as; normal healthy hepatocytes with a normal portal triad (Fig 4A). In addition; the heat stress-exposed rabbits showed moderate congestion in the hepatic sinusoid among the hepatic cords that surrounded the normal non-congested central vein (Fig. 4B).

Earlier studies showed prominent hepatic degeneration, hepatic vessel congestion, and perivascular leucocytic infiltration, in addition to a few degrees of focal necrosis were also detected in heat-stressed livers. These changes could be attributed to the hepatocytes' sensitivity to heat stress [45-46].

On the other hand; the *C. vulgaris*-treated rabbits (G3) exhibited a normal hepato-portal area with a normal portal triad surrounded by healthy hepatic cords (Fig. 4C). In addition, the *L.reuteri*-treated rabbits' liver (G4) illustrated normal hepatocytes with normal noncongested portal triad but also surrounded by mild periductular leucocytic infiltration (Fig 4D).

C.vulgaris has several antioxidant factors as α, β-carotene, α-tocopherol, ascorbic acid, and lutein that are active toward the free radicals and also interfere with the oxidation processes of lipid and cellular compartment [42]. So, *C.vulgaris* prohibit the hepatic toxicity, especially as a result of oxidative stress which exhibited as amelioration of oxidant/antioxidant hepatic condition and so explain its role against lipid peroxidation of liver tissues [47]

As shown in Table (6) the *L. reuteri* up-regulated the inflammatory cytokines in G4. Earlier study reported that *L.reuteri* either single or combined with other strains of lactic acid bacteria supplementation improved steroidogenesis, gametogenesis, and fertility by limiting the invasion of pathogenic bacteria and increasing anti-inflammatory agents, immunological responses, and reproductive hormones [40].

Spleen

As represented in Fig (5) the splenic tissue of both control, *C.vulgaris*-treated rabbits, and *L.reuteri*-treated rabbits exhibited normal lymphoid follicles fully packed with lymphocytes and a normal splenic artery (Fig. 5A, 5C, and 5D). On the contrary, the heat stress-treated rabbits illustrated moderate subcapsular lymphocytic necrosis with the existence of lymphocytic apoptotic bodies (Fig. 5B).

These findings could be attributed to; heat stress leading to metabolic and functional changes in various tissues. The produced free radicals (ROS) lead to apoptotic and degenerative changes [40].

Conclusion

Administration of *chlorella vulgaris* and *Lactobacillus reuteri* significantly improved the growth rate and FER of growing rabbits. Both of microalga and LAB enhanced ovarian function, and sex hormone secretion, and improved the general health condition, liver and kidney function, and oxidative and immunological status of animals. It can be recommended that; supplementation of rabbit foods by *C.vulgaris* and *L.reuteri* enhances the age of puberty in young rabbits.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study fulfilled the requirement and guidelines of good medical and laboratory practice (GCP and GLP) guidelines as well as institutional and animal care and use committee (LACUC) guidelines, recommendations and rules regarding the ethics of scientific research and approved on July 2020.

TABLE 1. The basal diet formulation for rabbits

Ingredients content	%	Chemical analysis	%
Barley	30.28	OM%	90.33
Clover hay (6%)	27.05	CP%	17.76
Wheat bran	18.40	CF%	11.5
Soybean meal (44% CP)	18.10	EE%	2.5
Molasses	3.00	NFE%	57.43
Di calcium phosphate	2.20	Ash%	9.66
NaCl	0.30	DE(kcal/kg)	2560
Premix (Vit. Min)*	0.30	Calcium	1.11
Limestone	0.22	Total phosphorus	0.85
DL-Methionine	0.10	Methionine+ cysteine.	0.65
Anticoccidia	0.05	Lysine	0.91
Total	**100**		

Dry matter (DM), Organic matter (OM), Crude protein (CP), Crude fiber (CF), Ether Extract (EE), and ash contents (Ash). Carbohydrates (nitrogen-free extract; NFE).

TABLE 2. Ambient temperature (C°), relative humidity (RH %), and THI values in different weeks of the experimental period under heat stress

	Heat stress conditions for G2, G3 and G4					
Week	**C°**		**RH%**		**THI**	
	Minimum	**Maximum**	**Minimum**	**Maximum**	**Minimum**	**Maximum**
1	19.29±0.42	35.14±0.5	22.86±2.20	61.86±1.4	29.38±0.55	32.51±0.55
2	19.86±0.96	40.86±1.79	24.57±3.83	66.43±1.97	28.36±1.54	37.06±1.54
3	18.86±0.26	36.29±0.89	31.43±4.63	72.71±3.51	28.65±0.72	32.38±0.72
4	17.14±0.34	34.86±0.26	31.29±0.28	66.86±2.25	30.14±0.16	31.08±0.16
	Normal conditions for G1					
	Minimum	**Maximum**	**Minimum**	**Maximum**	**Minimum**	**Maximum**
1	19.4±0.44	29.14±0.52	35.86±2.20	61.86±1.42	24.38±0.54	27.14±0.57
2	21.86±0.85	29.86±1.75	32.57±3.81	68.43±1.95	23.94±1.57	27.45±1.52
3	17.86±0.24	29.29±0.86	32.43±4.62	74.71±3.54	26.39±0.70	27.83±0.73
4	17.14±0.32	29.86±0.27	32.29±0.27	68.86±2.26	26.44±0.14	27.14±0.15

TABLE 3. Chemical analysis of *Chlorella vulgaris*

Chemistry	(gm/100 gm)
Carbohydrate	3.7
protein	60.5
Fat	12.8
Ash	4.6
Fiber	14
Moisture	5.4

TABLE 4. Minerals and traces in *Chlorella vulgaris*

Minerals and traces	Mg/Kg
Calcium	1820
Magnesium	1100
phosphorus	3550
Iron	230
Zinc	14
Copper	1.8
Selenium	1.3

TABLE 5. Antimicrobial activity of *Chlorella vulgaris* and *Lactobacillus reuteri*.

	Staph aureus	E coli	P aureoginosa
Chlorella	18	24	14
L. reuteri	13	16	12
Novobiocin	24	22	18
Tetracyclin	20	18	18

TABLE 6. Effect of using *Chlorella vulgaris* and *Lactobacillus reuteri* on blood biochemical parameters of rabbits under heat stress

	Control	Heat stress	Ch vulgaris	L. reuteri	P value
Albumin (g/dl)	$2.51+0.05^{b}$	$2.30+0.036^{b}$	$2.99+0.21^{a}$	$2.48+0.17^{b}$	0.004
Creatinine (mg/dl)	$0.525+0.11^{b}$	$1.86+0.13^{a}$	$1.34+0.06^{a}$	$1.71+0.34^{a}$	0.000
AST (U/L)	$14.56+0.54^{b}$	$24.99+4.47^{a}$	$11.29+0.52^{b}$	$18.19+2.05^{b}$	0.001
ALT (U/L)	$3.82+0.16^{bc}$	$6.81+1.18^{a}$	$1.69+0.31^{c}$	$4.46+0.85^{ab}$	0.002
Catalase (IU/L)	$346.1+29.68^{b}$	$80.70+31.82^{c}$	$434.45+35.75^{a}$	$276.77+17.88^{b}$	0.000
SOD (U/ml)	$187.5+42.13^{a}$	$137.5+77.6^{b}$	$250.00+26.65^{a}$	$187.50+18.84^{a}$	0.000
MDA(nmol/ml)	$9.76+0.35^{a}$	$17.31+0.52^{b}$	$8.74+0.58^{ab}$	$7.27+0.43^{b}$	0.002
P4 (ng/ml)	$0.75+0.19^{b}$	$0.75+0.15^{b}$	$0.47+0.06^{b}$	$1.61+0.33^{a}$	0.005
LH (ng/ml)	$0.25+0.05^{bc}$	$0.09+0.01^{c}$	$0.43+0.09^{ab}$	$0.63+0.07^{a}$	0.000
FSH (ng/ml)	$1.47+0.14^{b}$	$1.95+0.41^{b}$	$3.07+0.67^{b}$	$4.30+0.89^{b}$	0.000
IL-1 (pg/ml)	$4.04+0.47^{ab}$	$5.07+0.45^{ab}$	$2.19+0.34^{c}$	$3.59+0.55^{bc}$	0.000
TGFβ2 pg/ml	$45.92+3.51^{a}$	$13.60+2.22^{b}$	$19.37+6.15^{b}$	$38.22+10.02^{a}$	0.000
IF-γ (pg/ml)	$2.82+1.05^{b}$	$10.48+1.91^{ab}$	$16.19+4.68^{a}$	$17.31+4.02^{a}$	0.012
TNF-α (pg/ml)	$1.93+0.31^{b}$	$1.25+0.09^{b}$	$1.03+0.30^{b}$	$3.05+0.31^{a}$	0.001

Data were expressed as Mean ± SE. Means with different superscripts (a, b, c) within row differ significantly at $P<0.05$

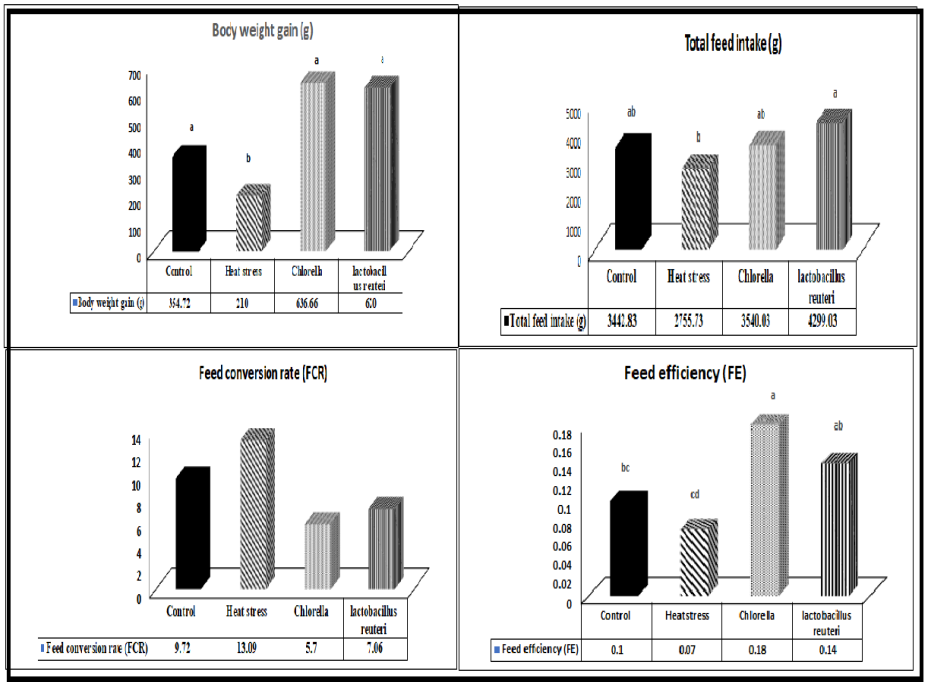

Fig. 1. Effect of using *Chlorella vulgaris* and *lactobacillus reuteri* on growth performance of rabbits under heat stress.

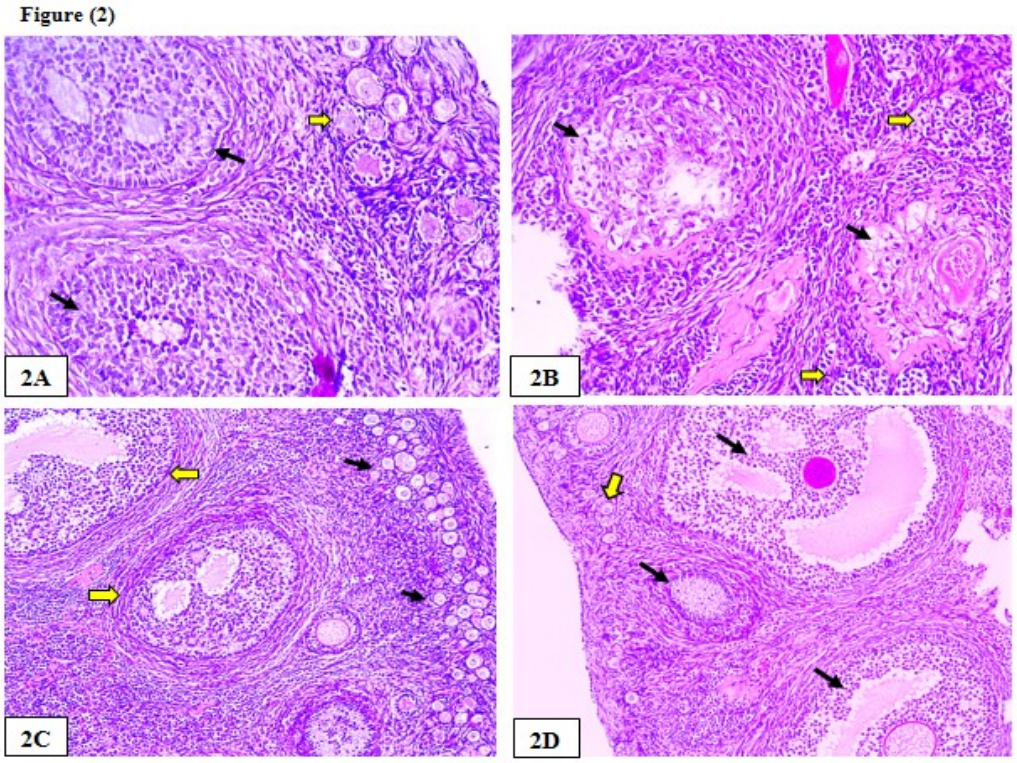

Fig. 2. The ovarian tissue changes in the different experimental groups. 2A: The ovary of control rabbits (G1) showed normal tissue architecture containing moderate numbers of the different follicular stages as mature follicles (black arrows) and primordial follicles (yellow arrows) H&E X 200. **2B:** The heat stress-treated rabbits (G2) illustrated severe degeneration and necrosis of multiple mature follicles (black arrows) adjacent to other atretic follicles (yellow arrows) H&E X 200. **2C:** *C.vulgaris*-treated rabbits (G3) displayed moderate follicular activity as the existence of a moderate number of the different stages of ovarian follicles as primordial follicles in the periphery (black arrows) adjacent to other mature follicles (yellow arrows) H&E X 100**. 2D:** The *L.reuteri*-treated rabbits (G4) exhibited favorable follicular activity where a small number of the primordial follicles were seen (yellow arrow) associated with the presence of a moderate number of mature follicles (black arrows) H&E X 100**.**

Figure (3)

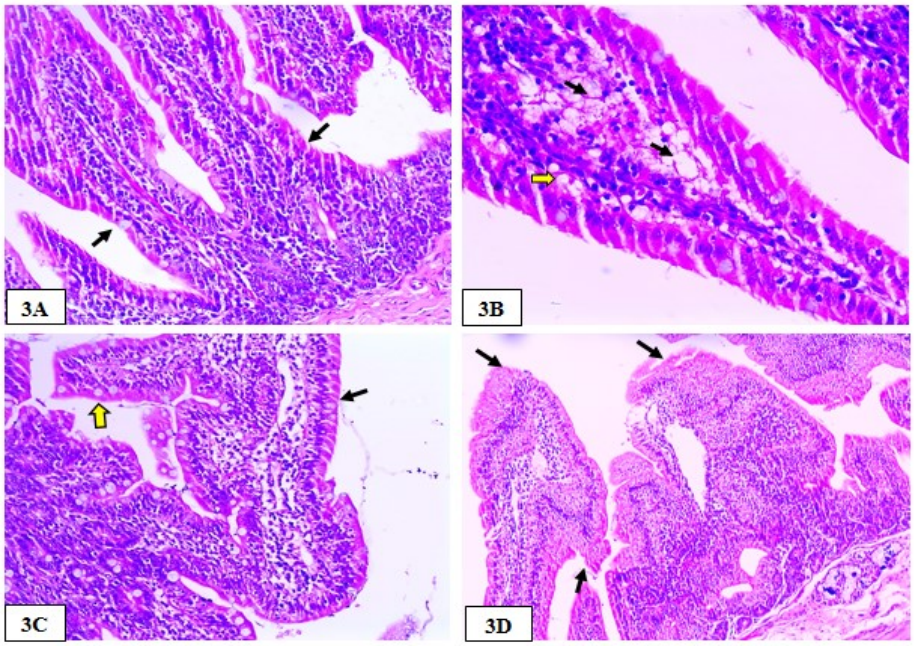

Fig. 3. The intestinal tissue changes in the four experimental groups. 3A: The intestine of control rabbits (G) exhibited normal intestinal villi with intact columnar epithelium and normal tissue cores (arrows) H&E X 200. **3B:** The heat stress-exposed rabbits (G2) showed degeneration and necrosis in the tissue core of the intestinal villi (black arrows) associated with vacuolar degeneration in some of the lining epithelium of the intestinal villi (yellow arrow). H&E X 400. **3C:** The *C.vulgaris*-treated rabbits **(G3)** displayed healthy intact intestinal villi lined with normal intact simple columnar epithelium (black arrow) with active goblet cells (yellow arrow) H&E X 200. **.3D:** The *L.reuteri*-treated rabbits **(G4)** illustrated great thickening in the intestinal villi due to severe hyperplasia in the villi lining epithelium (black arrows) H&E X 100.

Figure (4)

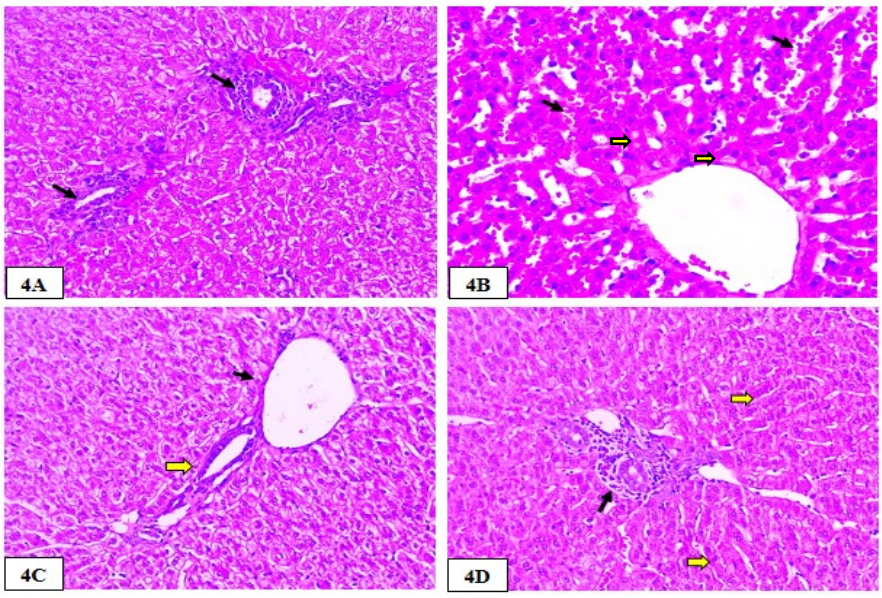

Fig. 4. The hepatic tissue changes in the four experimental groups. 4A: The liver of the control rabbits (G1) illustrated normal hepatic tissue with normal intact branches of bile ductules associated with mild periductular infiltrations of inflammatory cells (arrows) H&E X 200. **4B:** The heat stress-exposed rabbits (G2) showed mild focal peri-venular vacuolar degeneration adjacent to the central vein (yellow arrow) and moderate sinusoidal congestion among the hepatic cords (black arrows). H&E X 400. **.4C:** The *C.vulgaris*-treated rabbits (G3) displayed normal hepato-portal area with normal noncongested portal vein (black arrow) and normal intact bile ductules (yellow arrow) surrounded with normal healthy hepatic cords H&E X 200. **4D:** The *L.reuteri*-treated rabbits (G4) exhibited normal hepatocytes (yellow arrows) with normal bile ductules surrounded with mild periductular leucocytic infiltration (black arrow) H&E X 200.

Figure (5)

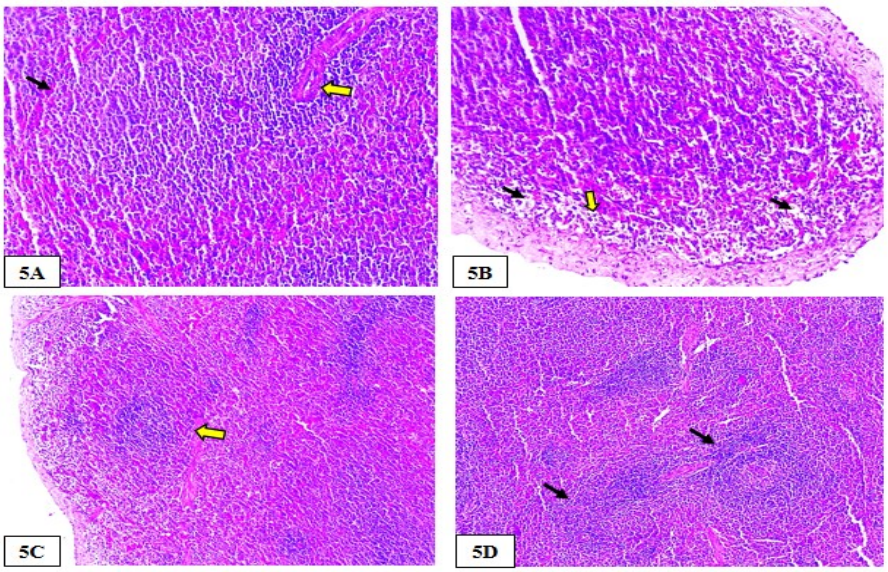

Fig. 5. The splenic tissue changes of the four experimental groups. 5A: Spleen of the control rabbits (G1) displayed normal active lymphoid follicles (black arrow) with normal splenic artery (yellow arrow). H&E X 100. **5B:** The heat stress-treated rabbits (G2) illustrated subcapsular lymphocytic necrosis (black arrows) and lymphocytic apoptotic bodies (yellow arrow). H&E X 100. **5C:** The *Chlorella vulgaris*-treated rabbits (G3) exhibited normal splenic tissue with normal lymphoid follicles in the white pulp (arrow). H&E X 100. **5D:** The *Lactobacillus reuteri*-treated rabbits (G4) exhibited the same tissue architecture of normality as, normal lymphoid follicles in the white pulp (arrows). H&E X 100.

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كلوريلا فولجاريس وبكتريا اللاكتوباسيلس ريتورى لتحسين سن البلوغ عند الأرانب تحت الإجهاد الحراري

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الملخص

الأرانب حساسة للغاية للإجهاد الحراري (HS) بسبب عدم وجود غدد عرقية لها لذلك يؤثر الإجهاد الحراري سلبًا على كميه العلف المستهلكة ومعدل النمو والكفاءة التناسلية. وإفراز الهرمونات. كلوريلا فولجاريس (*C.vulgaris*) هي طحالب دقيقة ذات قيمة غذائية عالية. كما تنتج بكتريا اللاكتوباسيلس ريتوراى (*L.reuteri*) كمية عالية من إنزيم الفيتيز الذي يحرر الفوسفور والمعادن الأخرى الضرورية للصحة من شكلها المرتبط . لذلك تم اقتراح أن كلا من طحلب الكلور يلا و بكتريا (*L.reuteri*) سوف تحسن الحالة التأكسدية والتناسلية للأرانب الصغيرة. بعد تحضير المستخلص الإيثانولي لطحلب الكلور يلا (*C.vulgaris*) وعدد مستعمرات بكتريا (*L.reuteri*) بلغ 1.5×105 CFU/ml والتي من المفترض أنها تنتج 100 وحدة من إنزيم الفيتيز (FTU). قمنا بتقييم النشاط المضاد للميكروبات لكل من الطحالب والبكتيريا ضد البكتيريا المسببة للأمراض. و تم استخدام ثمانية وعشرون أرنب نيوزيلاندي بوزن 1500.00جرام .في هذه الدراسة تم تقسيم الأرانب إلى أربع مجموعات متساوية (7 لكل مجموعة). المجموعة الأولى (G1) تمت تغذيتها على علائق تجارية (CD) ولم تتعرض للإجهاد الحراري وتحفظ في غرفة مكيفة. المجموعة الثانية (G2) تمت تغذيتها على علائق تجارية وتتعرض للإجهاد الحراري . المجموعة الثالثة (G3) تمت تغذيتها على علائق تجارية وتم اعطائها معلق الكلور يلا (*C.vulgaris*) بواسطة أنبوبة المعدة 60 ملغم / أرنب / يوم لمدة 4 أسابيع. المجموعة الرابعة (G4) تم تغذيتها على علائق تجارية وتم اعطائها بكتريا (*L.reuteri*) والتي أعطت إنزيم الفيتيز 250 (FTU) وحدة / أرنب / يوم لمدة 4 أسابيع. تم تسجيل الوزن الحي وتناول العلف أسبوعيا. تم حساب معدل و كفاءة التحويل الغذائي . تم تقدير نسبه كلا من الألبومين، الكرياتينين، ALT، AST Catalase، MDA، البروجسترون، FSH، LH، IL-1، TNFα، INFϒ، TGFB2) في البلازما. تم إجراء الفحص الهستولوجى للمبيض والكبد والأمعاء والطحال. أظهرت النتائج أن كلا من *C.vulgaris* و *L.reuteri* أدى الى زيادة معدل النمو والكفاءة التناسلية.

الكلمات الدالة : طحالب ، بكتريا ،البلوغ ،النمو والمبيض.