2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(4): 600-610.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj68
PII: S232245682400068-14

Physiological and Productive Responses to Dietary Supplementation of *Chlorella vulgaris* in Growing Rabbits

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ORIGINAL ARTICLE
Received: October 14, 2024
Revised: November 16, 2024
Accepted: December 09, 2024
Published: December 30, 2024

ABSTRACT

Chlorella vulgaris (CV) acts as an immuno-modulator and growth enhancer, however, studies were concerned about its impact on growing rabbits. The present study was undertaken to evaluate CV addition on physiological responses and productive performance (feed intake, feed conversion ratio, body weight, mortality rate, and other parameters) of APRI rabbits. A total of 45 growing rabbits at their weaning age with an initial body weight of 574.8 ±11.79g were investigated. The rabbits were divided into three equal groups; the first group (G1) received a basal diet without any additions. While, the other two groups, G2 and G3 received basal diets that contained 0.5g and 1.0g CV/kg diets. The results revealed the positive impact of CV on immunity (IgA, IgM, and IgG) status, especially IgG, which was significantly higher in G3 than in G1. No negative effects of CV on kidney and liver functions, since the lowest levels of creatinine, blood urea, aspartate aminotransferase, and alanine aminotransferase were recorded in G3 compared with G1. Throughout the experimental period (8 weeks), G3 was the best group in feed intake with the lowest feed conversion ratio reflected on achieving the highest body weight compared to other experimental groups. No mortality cases were recorded in G3, while, G1 and G2 almost showed the same mortality rate (%). The histopathological examination of rabbits' intestines indicated that a less inflammation presence of rabbit intestinal cells has been noticed in G3 compared to G1 and G2. Therefore, it could be concluded that using CV at a level of 1.0 g/ kg in diet is the best level that can be used as a natural feed additive. This contributes to the health of growing rabbits by protecting their intestines against inflammation, lowering the mortality rate, and ultimately improving their overall productivity.

Keywords: APRI rabbit, *Chlorella vulgaris*, Intestinal histopathology, Physiological responses, Productive performance

INTRODUCTION

In developing countries, rabbit production is considered very important due to their unique features, such as the average of borne kits which is 40 kits/year versus one calf/year in the ruminant animals (Abdel-Rahman and Ashour, 2023). To maintain rabbit production sustainability, a critical period, that is the weaning period, must be given great attention. Rabbits at this period are facing multiple stressors, such as starting their gradual feeding on pellets that may cause digestive disorders. Additionally, they are vulnerable to immune dysfunction besides environmental stressors, including rising ambient temperature and increasing intensity of heat waves (Abdelnour et al., 2018; 2019). To overcome this critical period, natural feed additives, such as probiotics and phytogenic additives can promote rabbit growth and reduce the mortality rate around the weaning age (Abdelnour et al., 2019).

Recently, microalgae supplementation, including *Chlorella vulgaris* (CV, green algae), an unconventional source of animal feed additives has been used (Abu-Hafsa et al., 2021). It proved its incredible ability to boost animal performance by enhancing their growth rate, meat quality, immunity, and antioxidant status (Abdelnour et al., 2019). The CV contains many biological compounds, such as protein, carbohydrates, polyunsaturated fatty acids, polysaccharides, and phenolic compounds (Madeira et al., 2017; Abdelnour et al., 2019). Kang et al. (2013) and Tsiplakou et al. (2018) described CV in poultry and goats as an immuno-modulator and antimicrobial agent. Besides, CV is considered a rich protein source compared to soybean protein. It can yield 2.5-7.5 tons/hectare/year compared to 0.6-1.2 tons/ hectare/year in soybean (Abu-Hafsa et al., 2021).

To cite this paper: Ashour G, Barakat SA, Abd El-Azeem NM, Abdel Mageed EM, Younan GE, El-Sayed HGM, Morsy SH, and Abdel-Rahman SM (2024). Physiological and Productive Responses to Dietary Supplementation of *Chlorella vulgaris* in Growing Rabbits. *World Vet. J.,* 14(4): 600-610. DOI: https://dx.doi.org/10.54203/scil.2024.wvj68

Some studies have focused on the effects of CV on rabbits' productive performance, and studies concerning its impact on immunity are rare. In Egypt, Abdelnour et al. (2019) showed that adding CV to growing rabbits' diets resulted in improving their growth rate and health. Therefore, the current study evaluated the physiological responses and productive performance to dietary supplementation of CV in APRI-growing rabbits. In addition, the economic efficiency of this supplementation was determined.

MATERIALS AND METHODS

Ethical approval

All experimental procedures followed the guidelines of the Scientific Committee of Animal Production Research Institute, coded 010303429. The current study has been conducted in the Rabbit Research Unit in Shaka Research Station, Kafr El-Sheik governorate, which belongs to the Animal Production Research Institute (APRI), Agricultural Research Center, Dokki, Giza, Egypt.

Experimental design and animal management

A total of 45 APRI rabbit bucks at their weaning age (5 weeks) with a mean initial body weight (BW) of 574.8 ± 11.79g have been used. The rabbits were housed in galvanized wire batteries, well-ventilated and clean indoor cages. The feeding strategy was started gradually to prevent disorder in the digestive tract. The rabbits were divided into three equal groups, including 15 APRI rabbits. According to the study of De Blas and Mateos (2010), the first group (G1) received the basal balanced diet without supplementations (Table 1). The other two groups, G2 and G3 were supplied with the basal diet containing 0.5 and 1.0 g CV/kg, respectively based on Abdelnour et al. (2019) (Figure 1). Fresh and clean water (*ad libitum*) was provided freely all day time. The CV powder was obtained from the National Research Center, located in Dokki, Giza, Egypt. The feeding strategy started when the rabbits were 5 weeks old and lasted for 8 weeks. The experimental period was executed during October and November 2023.

Table 1. Feed ingredients and chemical analysis of the basal diet (Percentage of dry matter basis, as DM) provided to the APRI-growing rabbit during the experimental period

Ingredients	Percentage	Chemical analysis	(Percentage as DM)
Barseem hay	35.00		
Barley grain	25.60	Dry matter (DM)	87.57
Soybean meal (44%)	14.50	Crude protein (CP)	17.04
Wheat bran	20.50	Organic matter (OM)	81.55
Molasses	3.00	Crude fiber (CF)	13.38
Ginger powder	0.00	Ether extract (EE)	2.275
Limestone	0.40	Digestible energy (DE, kcal/kg)(2)	2402
Di-Calcium phosphate	0.20	Calcium(2)	0.795
DL-Methionine	0.20	Total phosphorus(2)	0.534
Salt	0.30	Methionine(2)	0.445
Mineral-vitamin premix(1)	0.30	Lysine(2)	0.805
Total	100		

(1): PESTMIX (Pestar company, China). Each 3 kg mixture contains: Vitamin D3 2200000 IU, Vitamin A 12000000 IU, Vitamin E 10000 mg, Vitamin B1 1000 mg, Vitamin K 2000 mg, Vitamin B2 4000 mg, Vitamin B12 10 mg, Vitamin B6 1500 mg, Pantothenic Acid 10000 mg, Niacin 20000 mg, Folic acid 1000 mg, Biotin 50 mg, Choline chloride 500 gm, Manganese 55000 mg, Selenium 100 mg, Iodine 1000 mg, Zinc 50000 mg, and carrier $CaCO_3$, 3000 gm. (2): calculated by following De Blas and Mateos (1998). DM: Dry matter

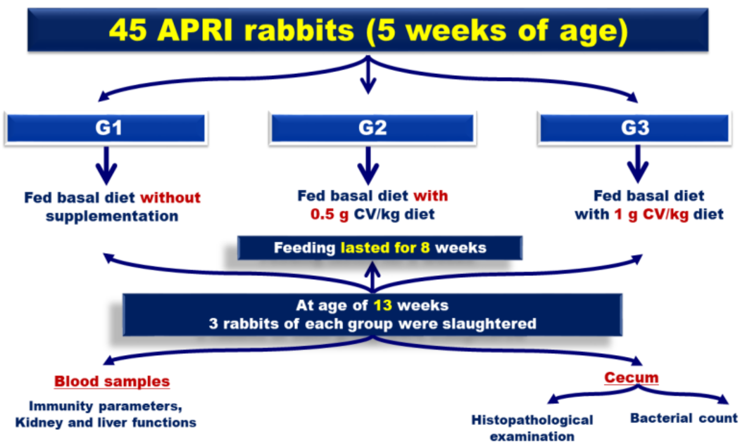

Figure 1. Experimental design of *Chlorella vulgaris* supplementation in growing rabbits' diet.

Productive performance

During the experiment, all weaned rabbits were weighed to record their initial body weight (IBW) and the final body weight (FBW). In addition, the rabbits' average daily body weight gain (ADG, g/day) feed intake (FI, g/d), and feed conversion ratio (FCR, g feed/g BW gain) were weekly recorded at 5-9, 9-13, and 5-13 weeks of their age. Besides, relative growth rate (RGR, %), performance index (PI, %), and mortality rate (MR, %) were calculated according to the following equations.

RGR (%) = FBW - IBW / ½ (IBW + FBW) x 100

FCR = (g) feed/ period / (g) ADG / during the same period

PI (%) = FBW / FCR x 100

MR (%) = Number of dead rabbits / Total number of rabbits at start x 100.

Feed analytical methods

Proximate analysis of the diets was carried out through the methods of AOAC (2007). Calcium was determined by an atomic absorption spectrophotometer. Phosphorous was determined calorimetrically using a spectrophotometer (ICP Optical Emission Spectrometer, Avio 220 Max, PerkinElmer company, USA).

Carcass traits

At the end of the experiment, 3 rabbits aged 13 weeks from each group were chosen randomly for slaughtering after 12 hours of fasting. Each rabbit was weighed before slaughtering. After slaughtering, the genital organs, urinary bladder, tail, and skin were removed. Then the data of carcass and its constituents as edible parts were considered for the following formula. The average carcass weight percentage for all three groups was 48.8%. The hot parts of the carcass, main body, giblets, heart, kidney, liver, and spleen were weighed as percent of slaughter weight. Furthermore, the percentages of the abdominal fat, and gastrointestinal tract (stomach, cecum, appendix, and large intestine) were also calculated. The pH of the stomach, small intestine, and cecum was measured by a pH meter (Model 20, Digital pH meter for Orion Research).

Carcass (%) = carcass weight × 100/live BW

Cecum bacterial count

Cecum bacterial count (total bacterial count, *coliform bacteria, and lactobacilli* × 10^6) were determined using viable plate count using serial dilutions of the samples containing viable microorganisms that were plated onto a suitable growth media. A one-gram cecum sample was incubated in Tryptone Soy Agar at 37°C for 24 hours. After that, 9 ml of normal saline was labeled in test tubes 10^{-1}, 10^{-2}, and 10^{-4}. Then, the nutrient agars, McConkey Agar, and MRS Agar plates from 10^{-3} to 10^{-4} were labeled. The samples were vortexed well to ensure bacterial distribution. After that, 1 ml of the sample was removed by a sterile pipette to transfer to a 10^{-3} dilution tube. The 10^{-1} was vortex well and transferred 0.1 ml to a 10^{-4} tube. These procedures were repeated until the transfer of 0.1 ml of 10^{-6} to tube 10^{-8} and vortexed again. The agar plates must be dried well and let it incubate for 2 days. After incubation, the incubated plates were transferred to light, and the colonies were checked by using a marking pen (APHA, 1960; Difco Manual, 1977).

Blood analyses

A total amount of 3 ml of blood samples were collected on the slaughtering day in sterile and heparinized tubes. The samples were centrifuged for 15 minutes at 3500 RPM, to get blood plasma and stored at -20°C until the evaluation of the following parameters, including immunological parameters (IgA, [ng/ml], IgM [pg/ml], and IgG [ug/ml]), kidney functions (creatinine [CR, mg/dl] and urea [mg/dl]), and liver enzymes (alanine aminotransferase [ALT, U/L] and aspartate aminotransferase [AST, U/L]).

All blood analyses were conducted utilizing kits provided by Bio-Diagnostic Company, located in Dokki, Giza, Egypt. All analyzing procedures were executed according to the manufacturer's pamphlets.

Histological features

When all contents from the cecum were evacuated completely, the cecum was washed with saline solution (0.9 % NaCl) and then dried well. Thereafter, specimens from the middle region of the cecum were taken to fix in 10% neutral formalin for 24 hours for histological examination. The histological procedures were carried out in the Animal Production Department, Faculty of Agriculture, Mansoura University, Egypt. According to Bancroft et al. (1990), the specimens were washed with running tape water, dehydrated in ascending grades of alcohol, cleared, impregnated, embedded in paraffin wax blocks, cut by microtone into thin sections (7-10 µm), and stained by hematoxylin and eosin.

After that, the stained sections were examined for histopathological features in the Animal Health Research Institute, Agricultural Research Center, Egypt (H & E, X400), to test which of the used doses of CV (as an anti-inflammatory agent) can reduce the inflammation that may occur in rabbit's intestine.

Economic efficiency

Based on the Egyptian market price in 2023, the economic efficiency of using CV in a rabbit's diet has been calculated. The price of each kilogram of live BW was 80 L.E.

Net revenue = Selling price – total feed cost

Statistical model

The differences among experimental groups were statistically analyzed using the general linear model procedures of SAS (2002), applying one-way analysis of variance (ANOVA). The following statistical model was used:

$Y_{ij} = \mu + T_i + e_{ij}$

Y_{ij} = The individual observation, μ = The overall mean, T_i = The fixed effect of the i^{th} treatments (i = 1, 2, 3), and e_{ij} = Random error associated with the individual. The mortality rate was analyzed by chi-square test. The differences among treatment means were separated according to Duncan's Multiple Range Test (Duncan, 1955). The significance level was set at 5%.

RESULTS AND DISCUSSION

Physiological responses

Immunological parameters

The effect of CV on growing rabbits' immunity parameters is illustrated in Table 2. The dose of 1.0 g CV/kg diet was more effective as an immune modulator compared to G2. The G3 (1.0 g CV/kg diet) recorded a significant ($p < 0.05$) elevation in the immunological parameters (IgM and IgG) than those recorded in G1 and G2. However, there were no significant ($p > 0.05$) differences among the experimental groups in the IgA values. G3 surpassed G1 in concentrations of IgA, IgM, and IgG by 4.2%, 13.3%, and 8.3%, respectively. Whereas, G1 and G2 showed almost similar and comparable values in all immunological parameters that were not statistically differed in the respective immunological parameters (Table 2, $p > 0.05$). According to the abovementioned results, we concluded that the greatest dose of 1.0 g CV/kg diet was more efficient in improving the immune status of growing rabbits than that of the lowest dose (G2) and the un-supplemented group (G1).

The obtained data disagreed with that of Abdelnour et al. (2019) who found that using CV at a level of 0.5 g/kg diet was better in enhancing immunity status than 1.0 g CV/kg in rabbits' diet. This difference could be attributed to the rabbit breed and the physiological and managerial conditions. The improvement in immunological parameters could be attributed to CV activities, such as anti-inflammatory anti-microbial, containing antioxidants, β-carotene, and vitamin B12 that can modulate immune functions (Abdelnour et al., 2019). A previous theory was performed by Safi et al. (2014), who proved that, carotenoid astaxanthin is present in CV, which enables CV to promote immunoglobulins production, through producing B cells in the gut-associated lymphoid tissue. Coelho et al. (2022) proposed that CV contains omega-3 fatty acids among the other biological compounds that confer to CV to act as an immune modulator and enhancer.

Immunoglobulins are released from B cells with a highly specific ability to bind to pathogens (antigens) and remove them from the body (Balan et al., 2019). The most significant immunoglobulin is IgG which represents 80% of the total immunoglobulins in the bloodstream. The IgG function neutralizes pathogens and promotes phagocytosis to remove them from the body (Borghesi et al., 2014). Immunoglobulins promote health through indirect ways, such as working on increasing growth factors and gut-weight. Additionally, they increase the beneficial bacteria and reduce the pathogenic bacteria in the gut (Balan et al., 2019). Therefore, the improved immunoglobulins in G3, specifically IgG mean increasing rabbits' ability to get rid of pathogens and have more resistance to diseases, which will reflect on their health and productive performance.

Kidney function

Verga (2002) confirmed that creatinine (CR) and blood urea (BU) are good indicators of kidney health and functions in rabbits. As can be seen in Table 2, there were no significant differences in both CR and BU concentrations between the control and treated groups ($p > 0.05$). However, the values of both indicators were numerically the lowest in G3 and the highest in G1. This emphasized that CV is a beneficial feed additive for rabbits with no negative effects on kidney functions. In addition, all values of both markers fell within the normal ranges as reported by Verga (2002), who illustrated that, CR normally ranged between 0.5-2.5 mg/dl. Meanwhile, the normal range of BU is 36.84-50.28 mg/dl in rabbits. The obtained results revealed that a rabbit diet containing 1.0g CV/kg succeeded in reducing values of both CR and BU more than the other doses. While, both G1 and G2 showed similar values of CR and BU.

The findings of the present study are in line with those of Hassanein et al. (2014), which compared adding of CV with spirulina in rabbits' diet. It was found that CV surpassed spirulina in reducing CR concentrations (0.99 mg/dl) in the supplemented group with CV versus 1.33 mg/dl in another comparative group.

Table 2. Effects of *Chlorella vulgaris* on immunological parameters, kidney functions, and liver enzymes of APRI-growing rabbits during the whole experimental period

Items		G1	G2	G3	SEM	P-value
Immunological parameters	IgA (ng / ml)	30.1	30.8	32.1	0.56	0.0936
	IgM (pg / ml)	38.0[b]	38.3[b]	43.4[a]	0.63	0.0024
	IgG (ug / ml)	16.0[b]	16.9[ab]	18.3[a]	0.49	0.0339
Kidney functions (mg/dl)	Creatinine	0.71	0.70	0.68	0.04	0.9347
	Urea	43.7	43.4	42.6	2.42	0.9417
Liver enzymes (U/L)	AST	40.3	39.3	38.3	2.17	0.8730
	ALT	46.8	45.0	44.3	2.85	0.7940

[a, b] Means in the same row with different superscript letters are significantly different ($p < 0.05$). G1: Control; G2: Group fed basal diet included 0.5 g CV/kg diet; G3: Group fed basal diet included 1.0 g CV/kg diet, SEM: Standard error of means

Liver function

In the present study, both AST and ALT enzymes were determined as important biological markers of liver health and functions. The effect of adding CV at two levels on AST and ALT is presented in Table 2. The activity of AST ascertained that the dose of 1.0 g CV/kg diet was more effective in reducing AST activity. The lowest value was recorded by in G3 (38.3 ± 2.17 U/L) that followed by G2 and both G2 and G3 were the lower groups in AST values compared with G1 with no significant differences ($p > 0.05$). However, it should be noted all the obtained values of AST are within the normal physiological range as reported by Verga (2002) which indicated AST ranges normally between 10 to 98 U/L in rabbit blood. The ALT showed the same results as AST, which was at the lowest level in G3 in comparison with the other experimental groups. The highest value (46.8 ± 2.85 U/L) of ALT was observed in G1 which proved the safety of CV, especially at the level 1.0 g/kg diet on liver enzymes. Verga (2002) recorded that its overall mean (in the three groups) was within the normal range and the normal level of ALT is 25 to 65 U/L with no significant differences among the experimental groups. Generally, the activity of ALT in rabbits is lower than the other species as noted by Verga (2002). The current results are compatible with the findings of Abdelnour et al. (2019). That previously confirmed the ability of CV (at a level of 1.0 g/kg diet to reduce live enzymes (AST and ALT), which reflects the possibility of supplementing rabbits' diet with CV with no harmful effects on rabbits' performance and health. A recent study used marine algae in rabbit diets and did not record any abnormalities or pathological responses to AST and ALT activities (Abu-Hafsa et al., 2021).

The importance of testing AST and ALT is clarifying the liver condition since AST is an enzyme that helps the body break down amino acids. Similar to ALT, AST is usually in the blood at low levels. An increase in AST levels may cause liver damage, liver disease, or muscle damage. The ALT is an enzyme found in the liver that helps convert proteins into energy for the liver cells. When the liver is damaged, ALT is released into the bloodstream and its levels increase Verga (2002).

Productive performance

Feed intake

Table 3 shows the effect of CV addition on the productive performance of APRI rabbits. It was noticed that FI was greater in G3 followed by G2 and both groups recorded the highest FI than that in G1. The findings confirmed that CV did not affect the feed palatability and encouraged rabbits to eat more. This is not consistent with the results previously reported by Sikiru et al. (2019) that used CV (at levels of 200 and 300 mg CV) in growing rabbit's diet and recorded a reduction in rabbits FI supplied with CV compared to the control group. Moreover, G3 was statistically ($p < 0.05$) different from G1 in FI during the experimental period. Additionally, FI was gradually increased during the 5-9 weeks of the study period in rabbits aged 9-13 weeks. However, the average FI during the experimental period (5-13 weeks) was lower than that recorded during 9-13 weeks.

Table 3. Effect of *Chlorella vulgaris* on productive performance of APRI-line rabbits

Parameters	G1	G2	G3	MES	P value
Feed intake (g/d)					
5-9 weeks	57.6^{b}	58.5^{a}	59.1^{a}	0.337	0.0080
9-13 weeks	93.9^{b}	95.0^{ab}	95.6^{a}	0.553	0.0485
5-13 weeks	75.7^{b}	76.8^{a}	77.4^{a}	0.318	0.0014
Feed conversion ratio					
5-9 weeks	2.4	2.4	2.3	0.048	0.2209
9-13 weeks	4.2^{a}	3.9^{b}	3.9^{ab}	0.085	0.0434
5-13 weeks	3.2^{a}	3.1^{ab}	3.0^{b}	0.050	0.0469
Initial body weight (g)	572.3	576.9	575.1	11.79	0.9702
Final body weight (g)	1884.7^{b}	1957.3^{a}	1989.7^{a}	19.52	0.0020
Average daily gain (g)					
5-9 weeks	24.1^{b}	24.6^{ab}	26.0^{a}	0.522	0.0515
9-13 weeks	22.8^{b}	24.7^{a}	24.6^{a}	0.441	0.0131
5-13 weeks	23.4^{b}	24.7^{a}	25.3^{a}	0.366	0.0027
Relative growth rate (%)					
5-9 weeks	74.3	75.0	77.5	1.529	0.3874
9-13 weeks	40.7	42.9	41.9	0.902	0.1747
5-13 weeks	106.9	109.1	110.4	1.350	0.2687
Performance index (%)	58.5^{b}	63.0^{a}	65.1^{a}	1.536	0.0110
Mortality rate (%)[(1)]	6.67	6.67	0	-	-

$^{a, b}$ Means in the same row with different superscript letters are significantly different ($p < 0.05$). G1: Control; G2: Group fed basal diet included 0.5 g CV/kg diet; G3: Group fed basal diet included 1.0 g CV/kg diet, SEM: Standard error of means, (1): Chi-square test

Feed conversion ratio

It was noted that G3 and G2 showed the best FCR during periods 5-9 and 9-13 weeks, and both groups (G2 and G3) recorded approximately the same values of FCR in the same periods. The detected reduction in FCR may be related to the amended digestive enzymes, in particular protease and lipase, leading to the promotion of intestinal efficacy (El-Basuini et al., 2023). Moreover, G3 was statistically different from G1 in FCR during the whole period (5-13 weeks, $p < 0.05$). Whereas, G3 was not significantly different than G1 during the other two experimental periods (5-9 and 9-13 weeks; $p > 0.05$). The enhancement of FCR emphasized the economic benefits of adding CV to growing rabbit diets. The obtained results of FCR are compatible with Sikiru et al. (2019) who, used different levels of CV, including 200, 300, 400, and 500 mg and found that the FCR was less in the supplemented group with 500 mg CV than the other doses. This confirmed the ability of CV to enhance rabbits' productivity.

Body weight and average daily gain

The enhancement in both FI and FCR has positively impacted APRI rabbit's BW, specifically FBW. As shown in Table 3, FBW was significantly elevated in the supplemented groups with CV in comparison with the control group ($p < 0.05$). The amount of increased FBW through the experimental period was 3.5, 3.4, and 3.2 times in G3, G2, and G1, respectively. This is considered evidence of the improved efficiency of feed utilization. In addition, G3 was the superior in ADG during the weeks 5-9 and 5-13. However, G3 was closer to G2 in ADG during the age of 9-13 weeks.

Relative growth rate, performance index, and mortality rate

To check the relative growth rate (RGR, %), adding CV in rabbits' diet insignificantly increased RGR (%) in the supplemented groups (G2 and G3) compared to G1 ($p > 0.05$). The G3 was the highest group in RGR at the age of 5-9 and 5-13 weeks. However, at weeks 9-13, G2 had the highest level of RGR. Furthermore, the treated groups significantly surpassed G1 in performance index (PI, %), and G3 was the higher one than the other two experimental groups $p < 0.05$). Interestingly, no mortality rate was recorded in G3, as all rabbits were in good health. While, the other two groups almost had equal (6.67) MR (%, Table, 3), which is considered an acceptable percentage for rabbits at this age as cleared by Rashwan and Marai (2000), who stated that MR (%) in the post-weaning period is less than 20% and the survival rate was ranged between 81-88%. The abovementioned results showed the improvement in rabbit immunity (particularly IgG) in G3, caused by CV addition, and made them more resistant to pathogens, leading to lower MR (%) as presented in

Table 3. Additionally, it could lead to suitable hygienic and managerial conditions for these rabbits. Besides the ability of this rabbit line (synthetic line, produced from crossbreeding between Red Baladi bucks with a dose of V-line) it is more adapted to the Egyptian environmental conditions.

The noticeable improvement of the productive performance (FBW, FI, FCR RGR, and PI) of the supplemented rabbits with CV, with special reference to the dose of 1.0 g CV/kg diet, could be attributed to the ability of CV to promote feed efficiency, nutrient digestibility, and absorption (El-Banna et al., 2005). The findings are in line with those of Abdelnour et al. (2019), Abu-Hafsa et al. (2021), El-Basuini et al. (2023), and Abdurakhmanova et al. (2024) which proved the enhancement in rabbits' productive performance in response to CV inclusion in their diets.

Carcass traits

Table 4 presents the rabbits that were fed with CV inclusion produced more carcass weight compared with the control group, particularly G3 which significantly differed from G1 ($p < 0.05$). The relative weight of abdominal fat, liver, kidney, heart, giblets parts, spleen, stomach, and pH of the stomach, small intestine, and cecum were similar in G2 and G3 and considered close to the obtained values in G1. Meanwhile, the three experimental groups (G1, G2, and G3) had the same value of spleen percentage. It is well known that the spleen is the largest secondary lymphoid organ that has a positive role in enhancing immunological and hematological parameters. In addition, it is the main filter for blood-borne pathogens and antigens, as well as, a key organ for iron metabolism and erythrocyte homeostasis (Bronte and Pittet, 2013; Kumari et al. 2019). Moller and Erritzoe (2000) reported that the spleen mass size is closely related to the level of humoral immunity, and a large spleen size will result in high humoral immunity. Meanwhile, the percentages of small intestine, cecum, and large intestine were lower in the treated groups, specifically in the rabbits fed with a 1.0 g CV/kg, with no significant differences ($p > 0.05$). These results agree with the findings of Abu-Hafsa et al. (2021), who used marine algae at a level of 4% per kg rabbit diet in New Zealand White and observed that percentages of liver, spleen, and kidney were lower in the rabbits who received CV in their diets than in the control group. They attributed their results to the lower fat content in rabbit carcasses.

Table 4. Effect of *Chlorella vulgaris* addition on carcass traits (%) and pH values of APRI-line rabbits

Carcass traits (%)	G1	G2	G3	SEM	P-Value
Carcass weight	47.5^{b}	49.2^{ab}	49.8^{a}	0.501	0.0474
Liver	3.4	3.6	3.7	0.161	0.5211
Heart	0.4	0.4	0.6	0.031	0.9759
Kidney	0.98	0.97	0.97	0.049	0.9819
Giblets Part	4.7	4.9	5	0.170	0.5680
Spleen	0.1	0.1	0.1	0.006	0.9785
Abdominal fat	0.7	0.7	0.6	0.045	0.8247
Gastrointestinal tract	22.5^{a}	20.3^{b}	19.8^{b}	0.493	0.0157
Stomach	4.9	4.3	4.3	0.220	0.1879
Small intestine	4.1	3.9	3.8	0.176	0.7420
Caecum	7.2	6.4	6.2	0.434	0.2627
Appendix	1.7	1.5	1.5	0.133	0.4045
Large intestine	2.0	1.7	1.7	0.158	0.3975
pH values					
Stomach	3.13	3.27	3.37	0.067	0.1199
Small intestine	7.40	7.43	7.57	0.058	0.5787
Caecum	6.23	6.27	6.50	0.116	0.3407

[a, b] Means in the same row with different superscript letters are significantly different ($p < 0.05$). G1: Control; G2: Group fed basal diet included 0.5 g CV/kg diet; G3: Group fed basal diet included 1.0 g CV/kg diet, SEM: Standard error of means

Cecum bacterial count

The effect of CV supplementation on cecal microbiota is illustrated in Table 5. Adding CV in growing rabbits' diet improved the cecum content, via increasing total bacterial count and *lactobacilli* bacteria and reducing the count of *coliform* bacteria, especially in G3 followed by G2. Both treated groups significantly differed from G1 in the measured microbiota as shown in Table 5 ($p < 0.05$). The elevation of the count of *lactobacilli* bacteria is a sign of enhancing feed digestibility and nutrient efficiency utilization (Phuoc and Jamikorn, 2017). This result is considered another explanation for the increase in FI and FCR, which was reflected in obtaining a higher FBW at the end of the experiment in treated groups compared to G1, as presented in Table 3. Whereas, the reduction in cecal *coliform* bacteria (the pathogenic bacteria) in the intestinal tract, leads to promote rabbits' health status and nutrient digestibility, thus improving their productive performance (Phuoc and Jamikorn, 2017). The findings are supported by Velankanni et al (2023), who found the positive effects of CV on animal immunity that are reflected in the gut microflora leading to the enhancement in animal health and nutrient unitization.

Histopathological features

The histopathological examination of growing rabbits' intestinal cells is shown in Figure 2. In the control group (Figures 2A, B, and C), a severe presence of sloughing tips of the intestinal villi and infusion of mononuclear inflammatory cells have been noticed with severe necrotic changes in the tips of some villi as well. Meanwhile, Figures 2D, E, and F (the photos of G2, the supplemented group with 0.5g CV/kg diet) reveal the moderate presence of sloughing and inflammation in the intestinal villi. A healthier presence of rabbit intestinal cells has been noticed in the photos of G3 (Figures 2O, H, and I). These photos illustrate a slight presence of inflammatory cells. In contrast, in Figure 2O, almost no inflammation or necrosis in the intestinal villi is observed. This means that supplemented rabbits with CV, especially with a 1.0 g / kg diet, succeeded in reducing the level of inflammation and necrosis from severe to slight level and preventing the inflammation as can be seen in Figure 2O. This could be an explanation for the elevated FBW in G3 compared to G1 and G2. As well as, the improvement in FCR, ADG, and RGR during the completely experimental period (5-13 weeks of rabbits' age). In addition, PI with zero MR % in G3 in comparison with G1 and G2 as discussed previously (Table 3).

These incredible results of CV, especially the higher dose of G3 (1.0 g/ kg diet) cloud be attributed to the anti-inflammatory activity of CV since it can reduce cytokines secretion that is associated with inflammatory activity. Furthermore, reducing the matrix called metalloproteinase, which causes tissue damage. In addition, in some species, CV might have a role in the growth and development of fibroblasts that are responsible for tissue repair (Coelho et al., 2022).

A lack of studies was concerned about the effect of CV on rabbit intestinal health except El-Basuini et al. (2023). The current data are supported by those of El-Basuini et al. (2023), who included CV in growing rabbit's diet at levels 3 and 5 g/kg diet for 8 weeks. The histological analysis of the rabbit's intestine revealed no pathological changes, such as necrosis or inflammation, when compared to the control group. This finding confirmed the health benefits of adding CV in growing rabbits' diets.

Table 5. Effect of *Chlorella vulgaris* on cecum microbiota of APRI-line rabbits

Parameters	G1	G2	G3	SEM	P-value
Total bacterial count ($x10^6$)	4.66^b	5.60^a	5.87^a	0.252	0.0317
Coliform bacteria ($x10^6$)	2.57^a	1.93^b	1.83^b	0.176	0.0411
Lactobacilli ($x10^6$)	1.33^b	2.57^a	2.63^a	0.176	0.0046

[a, b] Means in the same row with different superscript letters are significantly different ($p < 0.05$). G1: Control; G2: Group fed basal diet included 0.5 g CV/kg diet; G3: Group fed basal diet included 1.0 g CV/kg diet, SEM: Standard error of means

Table 6. Effects of experimental diets on economic traits of APRI-line rabbits at 13 weeks of age

Items	G1	G2	G3
Average feed intake (kg /head)	4.242	2.298	2.332
Price /kg diet (L.E.)	12.7	13.1	13.5
Total feed cost (L.E.)	53.9	56.3	58.5
Average weight gain (kg/head)	1.312	1.380	1.415
Selling price (L.E.)	105.0	110.4	113.2
Net revenue (L.E.)	51.1	54.1	54.7
Relative revenue (%)	100	105.9	107.1

G1: Control; G2: Group fed basal diet included 0.5 g CV/kg diet; G3: Group fed basal diet included 1.0 g CV/kg diet - Other conditions like management are fixed. - Ingredients price (Egyptian pound, EGP, per ton) at 2023 were: 14000 barley; 8000 barseem hay; 8000 wheat bran; 28000 soybean meal (44%); 500 limestones; 30000 premix; 80000 methionine; 10000 di-calcium phosphate; 8000 molasses; 500 salt; 800000 *Chlorella vulgaris*. 1 USD = 49.5 EGP

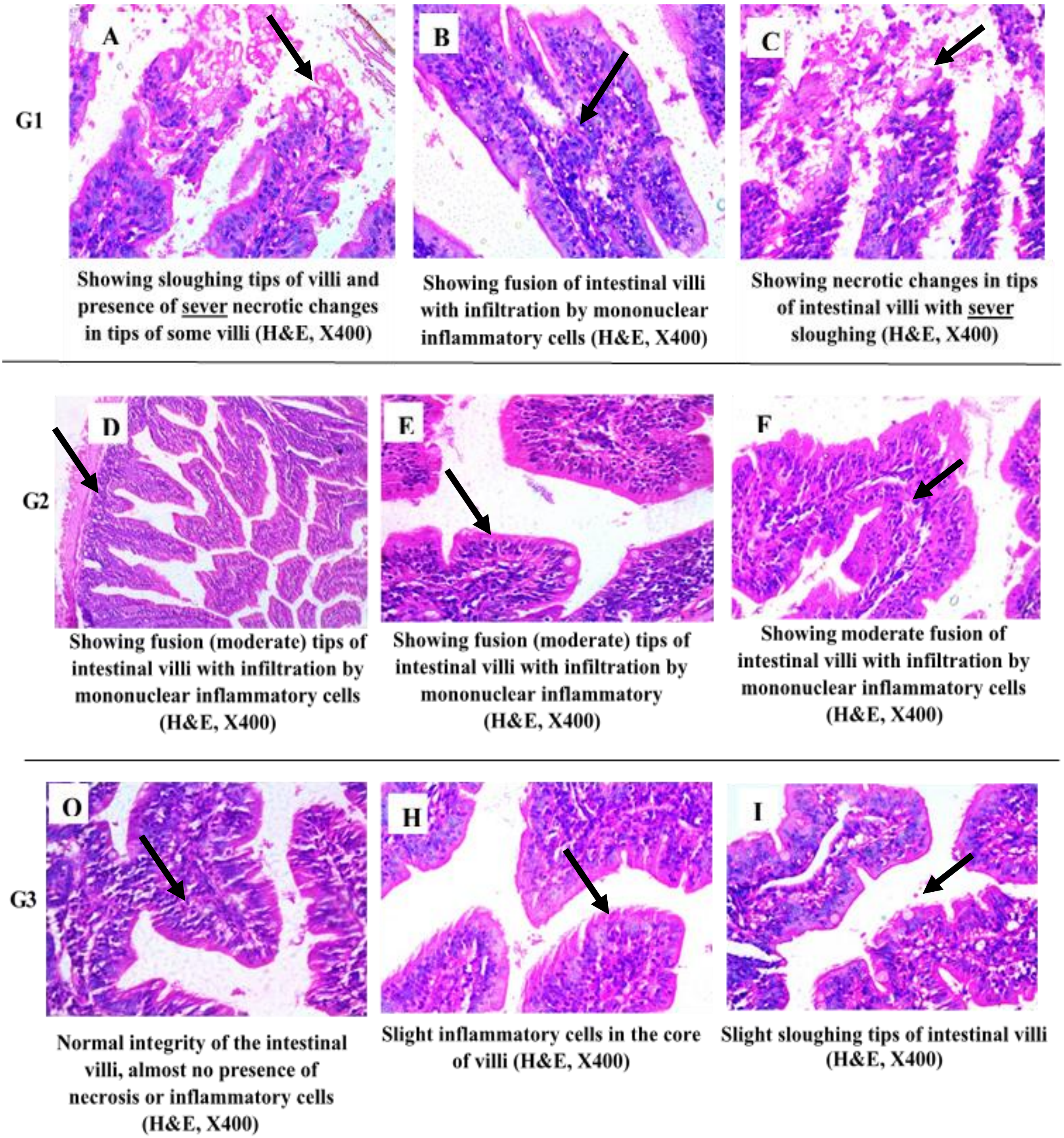

Figure 2. The examination of growing APRI rabbit's intestine at the age of 13 weeks. **G1:** Control; **G2:** Group fed basal diet included 0.5 g CV/kg diet; **G3:** Group fed basal diet included 1.0 g CV/kg diet.

Economic efficiency

The mentioned prices in Table 6 were reported based on the Egyptian market during 2023. Table 6 reveals that both treated groups recorded higher total feed costs ($p > 0.05$) in comparison with G1 due to CV addition. On the other side, CV supplementations, especially the level of 1.0 g CV/kg diet, succeeded in elevating the average weight gain and selling price, which led to increasing the net revenue and the relative revenue (Table 6). Referring to the positive benefits of CV on immunity and rabbits' productive performance with no hazards to their health, it is highly recommended to supplement rabbits' diets with CV and get economic benefits. Besides its health benefits through reducing the inflammation in intestinal villi confirmed by histopathological examination. The economic benefits of CV supplementation in rabbits' diet have been previously confirmed by Hassanein et al. (2014), who recorded higher net revenue and total weight gain in rabbits, despite increased total feed cost in treated rabbits compared with untreated ones.

CONCLUSION

The results of the present study confirmed the effectiveness of supplying growing rabbits' diet with CV, especially at a level of 1.0 g CV/kg diet. The dietary supplementation of CV could be severed as a natural and functional strategy that helps growing rabbits attenuate the shock of the post-weaning period. In the present study, adding CV at a level of 1.0 g/kg to the rabbits' diet improved immunity. In addition, it manifested health benefits through lowering levels of AST, ALT, CR, and urea in G3 as well as recording the highest final BW, with better FI and FCR in comparison to G1 and G2. The most significant result that has been discovered through the histopathological examination, proved the ability of CV (at a level of 1.0 g/kg diet) to attenuate the level of the sloughing and infusion of inflammatory cells in the intestinal villi from severe to slight level. This ability of CV is related to its property as an anti-inflammatory agent. Therefore, supplying growing rabbits with a 1g CV/kg diet is highly recommended, since CV is considered a natural protective agent for growing rabbits that can boost their health and productive performance, thus reducing the economic losses. Moreover, it is recommended to test higher doses of CV than 1.0 g/kg diets on intestinal cells especially in growing rabbits. Furthermore, evaluate these doses on the reproductive performance of both male and female rabbits.

DECLARATIONS

Acknowledgments

The authors gratefully acknowledge Prof. Dr. Nasser G. Fadel, Head of the Histopathology Department, Animal Health Institute, for his kindness, guidance, and support in the histopathological part of this current study

Authors' contributions

Prof. Dr. Gamal Ashour designed the study and drafted the manuscript, Prof. Dr. Safaa Atay Barakat and Dr. Noha Mahmoud Abdel-Azeem performed the statistical analysis and tabulation of the experimental data, Dr. Esraa Mohamed performed the blood chemical analysis, Dr. Shama hosy was responsible for the applying the experimental design, Prof. Dr. George Ezzat was responsible for the histopathological examination for rabbits intestine, Dr. Samah Mohamed Abdel-Rahman participated the blood chemical analysis and participated in writing the manuscript. Dr. Hazem Gaafar applied the bacterial count. All authors approved the final version of the manuscript before publishing in the present journal.

Competing interests

The authors have not declared any conflict of interest.

Funding

This study is funded in part by the Animal Production Research Institute.

Availability of data and materials

The collected data during the current study are available from the corresponding author upon reasonable request.

Ethical considerations

The authors confirmed that all authors have reviewed and submitted the manuscript and its original data to this journal for the first time.

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