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ORIGINAL ARTICLE

Comparative Effect of Probiotic and/or Canola Seed, Canola Meal and Canola Oil Dietary Supplementation on Growth Performance, Blood Component, Antioxidant Status and Immune Response in Growing Rabbits

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Abstract

OBJECTIVE: This study investigated the effects of probiotics-Progut (PG) and/or canola seed (CS), canola meal (CM), and canola oil (CO) on the growth performance, blood components, antioxidant status, and immune response in rabbits.
DESIGN: Randomized controlled study.
ANIMALS: One hundred twenty rabbits were used.
PROCEDURES: Rabbits were assigned into eight groups: control no supplemented group and the other supplemented groups including; PG, CS, CS + PG, CM, CM + PG, CO, and CO + PG. Growth performance, liver and kidney biomarkers, lipid profile, antioxidant markers, and immunological parameters were determined at the end of the study.
RESULTS: CM and CO had the strongest impact on growth performance, as they significantly increased body weight gain and reduced feed intake. PG significantly increased IgM and NO levels and decreased cholesterol level. CS and CM significantly increased leukocyte count, total protein and globulin levels. Additionally, CS increased serum lysozyme activity, whereas CM increased the hepatic glutathione (GSH) levels. CO increased ($P < 0.05$) the values of total protein, globulin, SOD, glutathione, and lysozyme and significantly diminished Malondialdehyde (MDA).
CONCLUSION AND CLINICAL RELEVANCE: Supplementation of CS with PG is recommended to obtain good outcomes on rabbits' general health, immunity, and performance without adverse effects on lipogram.

Keywords: Antioxidant status, Canola, Immune response, Probiotic, Rabbit

1. Introduction

Rabbit meat production can be a possible solution to meet the meat shortage in developing countries, especially Egypt [1]. Furthermore, its meat is the most delicious, low in fat and cholesterol (4 %), easily digestible, high in protein content (25 %), and low in caloric value (160 Kcal/100 g meat) [2]. Rabbits can utilize a wide variety of feed sources, as they are herbivorous monogastric animals [3]. Rabbits are sensitive to enteric diseases [2], there are many attempts to increase the immune response by using alternative feed additives to avoid the use of antibiotics to treat enteric diseases and as growth promoters that kill some of the gastrointestinal flora of rabbits [4]. Nonantibiotic feed additives used in the diet of rabbits for disease prevention improve growth performance, increase productive activity, and enhance carcass traits and meat production [5,6].

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Probiotics are among the most important feed additives used in farm animals. They are important for efficient digestion, maximum absorption of nutrients, and the maintenance of balanced intestinal microorganisms [7,8]. Probiotics also, enhance gut colonization and stabilize eubiosis through competitive growth against harmful microorganisms, decreasing intestinal pH, producing lactic acid, and emboldening digestion by producing vitamins and enzymes. This function strengthens the animals' new specific immune defenses [9].

Modern knowledge is applied to agricultural biotechnologies in cell and molecular biology to produce new varieties of similar genetic materials. The use of genetically modified crops was introduced in 1995, and has grown dramatically [10]. Biotechnology techniques have been used to improve the quality and performance of these crops [11]. In recent years, to address the raised costs of animal feeds, several experimental studies have been conducted to find an alternative feed to meet the needs of animals, as researchers have directed the introduction of genetically modified rapeseeds (Canola) in the animal diets. Most of these studies concluded that canola meal (CM) is a good nutritional source of feed value with a good amino acid balance and is recommended for use in animals that require intermediate levels of energy and high levels of methionine, cysteine, and histidine [12−14]. Canola is the recorded name of rapeseed containing less than 2 % erucic acid of the total fatty acids and less than 30 μmol alkenyl glucosinolates/gram of oil (free from seed dry matter of the seed) [15]. In the 1960-the 1970s permitting rapeseed oil became a vital food oil, and rapeseed meals became important as feed for livestock [16]. Rapeseed meal has been used in rabbit feed for a long time [17,18] and is still used in experimental and commercial diets [19,20]. It is recommended for Animals require intermediate levels of energy and high levels of methionine, cysteine, and histidine [14]. Therefore, this study was conducted to assess the influence of dietary supplementation with probiotics and/or canola seed (CS), canola meal (CM), and canola oil (CO) on growing rabbits as alternative sources of antibiotics.

2. Materials and methods

2.1. Probiotic

Hydrolyzed Brewer's yeast (Progut) was purchased from Hankkija Oy/Suomen Rehu (Hyvinkää, Finland).

2.2. Canola seed

CS were purchased from the market as seeds suitable for planting and processed to obtain other forms of the plant (canola meal and oil).

2.3. Animal and experimental design

One hundred twenty mixed-sex California rabbits were obtained from the Faculty of Agriculture, Mansoura University, at weaning age (average 4 weeks of age), with an average body weight of 618 g, and they were apparently healthy. Animals were provided with continuous feeders and automatic water. The rabbits were handled following animal welfare guidelines, and the protocol was approved by the Animal Ethical Committee of the Faculty of Veterinary Medicine, Mansoura, University, Egypt. After acclimatization, rabbits were divided into the following experimental groups: control group fed a control diet that covered the nutrient requirements of the growing rabbits [13], the Progut supplemented group (PG) (1.0 g/kg diet) according to Cetin et al. [21], the CS group supplemented with 15 % CS according to Castell and Falk [22], the CS + probiotic (Progut)-supplemented group (CS + PG), the canola meal group (CM) supplemented with 15 % canola meal according to Rabie et al. [23], CM + PG group, the CO supplemented group (1.5 %) according to Shahryar et al. [24], and CO + PG group. The compositions of the different experimental diets are shown in Table 1.

2.4. Samples collection

At the end of the experimental period (12 weeks of age), five animals from each experimental group were randomly selected, and two separate blood samples were withdrawn from the ear vein of each animal. The first blood sample was collected in coated tubes that containing di-potassium EDTA as anticoagulant and gently mixed for hematological estimation. The second sample was placed in a vacutainer tube and placed in an inclined position at room temperature for 20 min to clot, maintained under cooling in the refrigerator to complete clot formation, and centrifuged for 10 min at 3000 rpm to completely separate the serum. Serum was carefully transferred to 0.5 ml Eppendorf tubes then stored at at−20 °C for serum biochemical and immunological analysis. All animals were slaughtered in accordance with Islamic roles. Hepatic tissue was isolated, and 1.0 g was homogenized from

Table 1. The composition of the experimental diets.

Ingredient %	Control	Canola seed	Canola meal	Canola oil
Yellow corn	12.74	16.4	19.4	6.04
Soybean	12.7	7.74	4.74	12.4
Wheat bran	29	19.26	23.26	29.5
Alfalfa hay	28.96	25	21	33.96
Barley	12	12	12	12
Canola seed	–	15	–	–
Canola meal	–	–	15	–
Canola oil	–	–	–	1.5
Lime stone	1	1	1	1
Molas	3	3	3	3
Salt	0.3	0.3	0.3	0.3
Premix	0.3	0.3	0.3	0.3
Calculated analysis				
Digestible energy kcal/kg	2503	2502	2505	2499
Crude protein %	17.01	17.10	17.06	17.10
Crude fiber %	13.04	13.81	11.65	14.27
Ether extract %	2.72	6.51	4.25	4.09
Lysine %	0.79	0.76	0.71	0.80
Methionine %	0.22	0.26	0.22	0.22
Meth.+Cyst. %	0.55	0.65	0.58	0.55
Calcium %	0.88	0.86	0.85	0.94
Total phosphorus %	0.59	0.59	0.64	0.59

each rabbit for evaluation of antioxidant and oxidative stress parameters.

2.5. Growth performance

The live body weight (LBW) of growing rabbits was registered individually at the commencement of the trial and then at weekly intervals until the end of the trial. The feed intake (FI) and body weight gain (BWG) of growing rabbits were recorded weekly. Subsequently, the feed conversion ratio (FCR) was determined as the quantity of the diet consumed to gain a unit of body weight [23].

2.6. Serum biochemical analysis

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured using diagnostic kits from Colorimetric Biolabo (France). The total protein (TP) and albumin levels were estimated using commercial diagnostic kits (Spinreact, Spain). Creatinine was estimated using human kits (Germany), whereas urea was detected using Diamond (Egypt) kits. Glucose, cholesterol, triglycerides, and HDL levels were measured using ready-to-use kits supplied by Spinract. All parameters were spectrophotometrically detected (5010 photometer, BM Co., Berlin, Germany) using the enclosed pamphlets.

2.7. Measurement of hepatic oxidative stress and antioxidant markers

Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were detected colorimetrically by spectrophotometry using Bio-diagnostic kits (Egypt) according to the manufacturer's instructions.

2.8. Measurement of some immunological parameters

2.8.1. Total leukocytic count

Anticoagulated blood was used to count total leukocytes (TLC) [25].

2.8.2. Lysozyme activity

Serum lysozyme activity was determined using the turbidimetry method described by Hou et al. [26]. Lysozyme substrate suspension was prepared by adding 0.75 mg Lyophilized *Micrococcus Lysodeikticus* cells to 1 ml of sterilized PBS (PH 5.8), 175 µl of the prepared substrate solution was added to each well of a round bottom microtiter plate, and 25 µl of serum was added to each well. The absorbance was read at 450 nm immediately, and after 20 min, the decrease in absorbance was calculated. The lysozyme in serum, expressed as µg/ml, was obtained from lysozyme standard, which was constructed previously using Lyophilized Hen Egg-White Lysozyme (Sigma–Aldrich).

2.8.3. Estimation of serum nitric oxide

Nitric oxide (NO) was determined spectrophotometrically using a colorimetric assay (BM Co., Germany, 5010) using Bio-diagnostic kits [27].

2.8.4. Measurement of serum immunoglobulin M (IgM)

Immunoglobulin M was measured according to Kaplan et al. [28] by the immune-turbidimetric method using the kit provided by ROCHE Cobas (USA). The agglutination resulting from the antigen/antibody reaction (anti-IgM antibodies in the reagents that react with the antigen in serum samples) was measured turbidimetrically.

2.9. Statistical analysis

All data were analyzed using a statistical software program (SPSS for Windows, version 20, USA). Data are presented as the mean ± standard error of the experimental study. ANOVA was used to determine the differences between the means of

all treatments using Duncan's multiple comparison tests. Differences between the results were considered significant at P less than 0.05 [29]. Figures were generated using GraphPad Prism version 5 (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

3.1. Growth performance

Final body weight (FBW), BWG, and FCR were remarkably improved in the groups supplemented with CS + PG, CM, CM + PG, and CO. Meanwhile, there was no significant change in the other treatment groups (PG, CS, and CO + PG) ($P < 0.05$). In contrast, feed intake significantly declined in CO-supplemented rabbits. There were no significant changes in the other supplemented groups ($P < 0.05$) (Table 2).

3.2. Serum biochemical results

The serum values of ALT, AST, albumin, creatinine, urea, and glucose in all investigated groups were not significantly different from those in the control group ($P < 0.05$) (Table 3). In contrast, the CS-, CM-, CM + PG-, CO-, and CO + PG-supplemented groups revealed a significant increase in the total protein and globulin levels compared with the control group, and the highest increase in both parameters was recorded in the CM-supplemented group. Additionally, the PG- and CS + PG-supplemented groups showed insignificant changes in both parameters compared with the control group ($P < 0.05$) (Table 3).

3.3. Lipid profile

The serum cholesterol level was significantly elevated in the CO-supplemented group, whereas it was not significantly affected in the CS, CS + PG, CM, CM + PG, and CO + PG groups. Conversely, it was significantly reduced in the PG supplemented group compared with the control rabbit ($P < 0.05$) (Fig. 1). Triglyceride levels were significantly increased in the CS, CM, CM + PG, CO, and CO + PG supplemented groups, and the highest increase was recorded in the CM + PG supplemented group. Otherwise, there was no significant change in the PG and CS + PG-supplemented groups compared with the control group. The HDL level was not significantly different between the investigated groups (Fig. 1).

Table 2. Growth performance of growing California rabbits at eighth week postdietary supplementation with canola seed, canola meal, canola oil and/or probiotic (Progut).

Parameter	Treatment							
	Control	PG	CS	CS + PG	CM	CM + PG	CO	CO + PG
IBW g	619.25 ± 1.35	616.80 ± 1.78	616.88 ± 1.53	617.45 ± 3.26	618.15 ± 1.36	619.05 ± 1.56	618.71 ± 0.66	618.29 ± 1.41
FBW g	1833.60 ± 17.03^{d}	1877.80 ± 12.18^{cd}	1872.26 ± 32.97^{cd}	1959.60 ± 48.52^{abc}	2017.00 ± 34.99^{ab}	2042.93 ± 35.20^{a}	2023.53 ± 45.30^{a}	1926.55 ± 17.62^{bcd}
BWG g	1214.36 ± 18.03^{d}	1261.00 ± 13.34^{cd}	1255.38 ± 32.64^{cd}	1342.16 ± 49.90^{abc}	1398.86 ± 34.06^{ab}	1423.88 ± 36.75^{a}	1404.82 ± 45.23^{a}	1308.26 ± 17.01^{bcd}
FI g	3707.9 ± 54.4^{ab}	3747.0 ± 55.2^{ab}	3805.2 ± 81.0^{a}	3735.6 ± 77.6^{ab}	3573.4 ± 52.1^{bc}	3686.4 ± 85.0^{ab}	3472.4 ± 59.7^{c}	3880.8 ± 92.7^{a}
FCR	3.06 ± 0.07^{a}	2.97 ± 0.03^{ab}	3.04 ± 0.07^{a}	2.80 ± 0.13b^{c}	2.56 ± 0.07^{d}	2.60 ± 0.12^{cd}	2.48 ± 0.04^{d}	2.97 ± 0.05^{ab}

BWG, body weight gain; **CM**, (Canola meal); **CM + PG**, (Canola meal + probiotic-Progut®); **CO**, (Canola oil); **CO + PG**, (Canola oil + probiotic Progut).Data are expressed as mean ± standard error of the mean ($n = 5$). Different letters indicate significant differences between groups (P less than 0.05); **CS**, (Canola seed); **CS + PG**, (Canola seed + probiotic-Progut); *FBW*, Final body weight; *FCR*, feed conversion ratio; *FI*, feed intake; *IBW*, Initial body weight; **PG**, (probiotic-Progut).

Table 3. Serum biochemical parameters in California rabbits at eighth week postdietary supplementation with canola seed, canola meal, canola oil and/or probiotic (Progut).

Parameter	Treatment							
	Control	PG	CS	CS + PG	CM	CM + PG	CO	CO + PG
ALT U/L	11.8 ± 0.58^{ab}	11.5 ± 0.82^{b}	11.18 ± 0.50^{b}	11.75 ± 0.97^{ab}	11.00 ± 0.71^{b}	12.00 ± 0.82^{ab}	13.62 ± 0.72^{a}	11.18 ± 0.57^{b}
AST U/L	13.27 ± 0.93	13.05 ± 0.63	11.65 ± 0.68	12.71 ± 0.66	11.74 ± 0.36	12.94 ± 0.66	11.82 ± 0.71	12.86 ± 0.69
TP g/dl	6.59 ± 0.33^{d}	8.17 ± 0.51^{cd}	10.33 ± 0.68^{ab}	8.00 ± 0.41^{cd}	10.88 ± 0.33^{a}	9.34 ± 0.30^{abc}	8.92 ± 0.44^{bc}	8.49 ± 1.02^{bc}
Albumin g/dl	4.32 ± 0.10	4.72 ± 0.45	4.89 ± 0.12	4.21 ± 0.49	4.82 ± 0.51	4.60 ± 0.35	4.08 ± 0.49	4.20 ± 0.19
Globulin g/dl	2.27 ± 0.37^{d}	3.45 ± 0.48^{cd}	5.44 ± 0.69^{ab}	3.78 ± 0.41^{cd}	6.06 ± 0.50^{a}	4.74 ± 0.12^{abc}	4.84 ± 0.44^{abc}	4.29 ± 1.01^{bc}
A/G ratio	2.16 ± 0.4^{a}	2.05 ± 0.6^{ab}	1.39 ± 0.27^{abc}	1.22 ± 0.27^{abc}	0.83 ± 0.13^{bc}	0.98 ± 0.09^{abc}	0.72 ± 0.19^{c}	2.06 ± 0.76^{ab}
CRE mg/dl	0.76 ± 0.04	0.81 ± 0.03	0.79 ± 0.07	0.82 ± 0.02	0.75 ± 0.04	0.82 ± 0.04	0.70 ± 0.10	0.69 ± 0.02
Urea mg/dl	30.42 ± 1.57	27.89 ± 0.91	28.79 ± 1.82	26.38 ± 1.82	27.78 ± 1.11	26.00 ± 1.82	27.5 ± 1.06	27.66 ± 1.99
Glucose mg/dl	98.27 ± 5.72	94.38 ± 4.3	92.32 ± 5.04	93.36 ± 4.26	93.22 ± 4.26	92.36 ± 4.18	90.83 ± 4.5	90.07 ± 4.36

A/G ratio, albumin/globulin ratio; *AST*, Aspartate aminotransferase; **CM**, (Canola meal); **CM + PG**, (Canola meal + probiotic-Progut); **CO**, (Canola oil); **CO + PG**, (Canola oil + probiotic-Progut). Data are expressed as mean ± standard error of the mean ($n = 5$). Different letters indicate significant differences between groups (P less than 0.05). *ALT*, alanine aminotransferase; *CRE*, creatinine; **CS**, (Canola seed); **CS + PG**, (Canola seed + probiotic-Progut); **PG**, (probiotic-Progut); *TP*, Total Protein.

3.4. Hepatic oxidative stress and antioxidant markers

As shown in Fig. 2, the hepatic MDA level was significantly decreased in the CS + PG, CM + PG, CO, and CO + PG groups, and a significant reduction was recorded in the CO and CO + PG groups. There was no significant difference in the PG-, CS-, and CM-supplemented groups compared with the control one ($P < 0.05$). Additionally, there was a significant increase in SOD activity in the CS + PG-, CO-, and CO + PG-treated groups. Moreover, there was no significant change in the other supplemented groups compared with that in the control group. In contrast, catalase activity exhibited insignificant changes in all experimental groups compared with the control one. GSH level was significantly increased in all supplemented groups compared with the control group, except in the PG- and CS-supplemented groups it was insignificantly varied. In addition, the highest increase was recorded in the CO + PG supplemented group compared with all other groups ($P < 0.05$).

3.5. Total leukocytic count and serum immunological results

The CS, CS + PG, CM, CM + PG, and CO + PG supplemented groups showed a significant increase in the TLC compared with the control group, whereas in the PG and CO supplemented groups, TLC did not differ significantly, as illustrated in Fig. 3 ($P < 0.05$). There was a significant increase in IgM in the PG-supplemented group compared with the control group, while in the CS + PG CM + PG and CO + PG treated groups it was insignificantly changed. Moreover, IgM was significantly decreased in the CS-, CM-, and CO-supplemented groups compared with the control group. In addition, serum lysozyme activity was significantly increased in the CS, CS + PG, CM + PG, and CO supplemented groups and was not significantly affected in the PG, CM, and CO + PG supplemented groups compared with that in the control rabbits. Serum nitric oxide level was significantly increased in the PG, CS + PG, and CM + PG supplemented groups compared with those in the control. Furthermore, there was no significant change in the CS-, CM-, CO-, and CO + PG-supplemented groups compared with the control animals.

4. Discussion

Our research estimated the effects of dietary supplementation of CS, CM, CO, and/or PG on the growth performance and hemo-biochemical, antioxidant, and immune response parameters in growing California rabbits. Rabbits supplemented with probiotics exhibited insignificant changes in growth performance (FBW, BWG, FI, and FC), which parallels Fathi et al. [30] in rabbits supplemented with probiotics for 8 weeks at doses of 200 and 400 g probiotic/t feed. Meanwhile, Ezema and Eze [31] and Ayyat et al. [32] observed a significant increase in body weight gain in rabbits supplemented with 0.12 g yeast/kg of diet for 13 weeks, or with 1 g probiotic (Bactocell) for 8 weeks, respectively.

The growth-promoting effects of CS + PG, CM, CM + PG, and CO on FBW and BWG of rabbits could be due to the high levels of free, unsaturated, and omega-3 FA in canola, which have the main effect on optimal fat metabolism and hence body weight [33]. Our data agreed with Ibrahim et al. [34], who observed a significant increase in FBW and

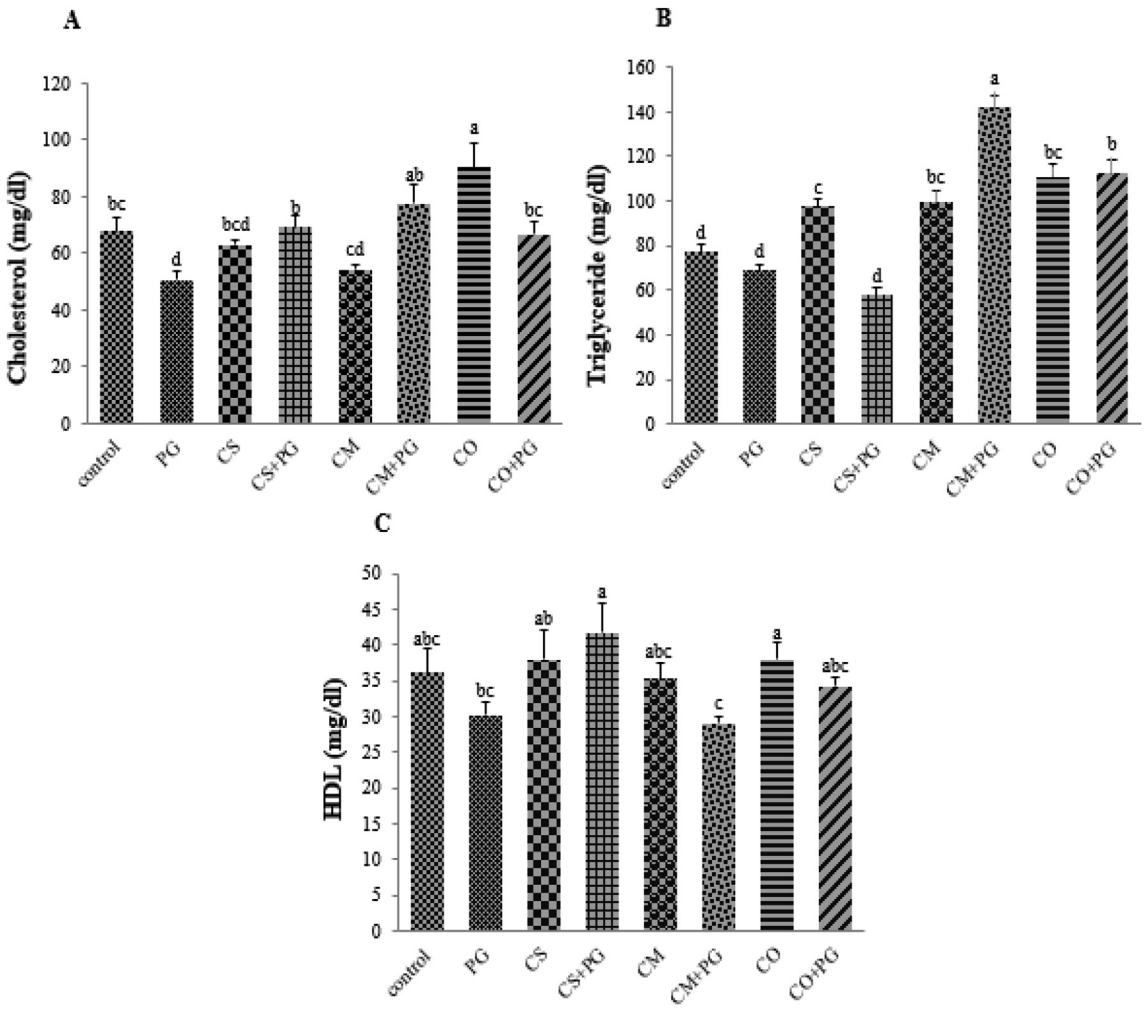

Fig. 1. Lipid profile (a) cholesterol (b) triglyceride (c) HDL in California rabbits at eighth week postdietary supplementation with canola seed (CS), canola meal (CM), canola oil (CO)and/or probiotic (PG).

BWG in rabbits supplemented with 25, 50, 75, and 100 % CM as replacement for SBM in for 9 weeks. Also, Hemid et al. [35], reported a significant increase in LBW and BWG in rabbits supplemented with 33 % and 66 % canola for four weeks. Similarly, CO significantly improved the average daily gain in rabbits supplemented with 10 g canola oil/kg diet for 6 weeks [36].

We recorded an improvement in the FCR in the previously mentioned groups, that may be due to the high levels of essential and unsaturated fatty acids. Malabsorption of FA in canola could play a major role in FCR by reducing the rate of feed passage through the digestive tract, which allows greater absorption of all nutrients in the diet [37]. This in line with Ibrahim et al. [34], Rahimi et al. [37], and Nobakht et al. [38], who showed that FCR significantly improved in rabbits or in broiler chicks fed diet supplemented with CM, respectively. Since, Xiccato [39] recommended that nutrient digestibility increased when fat was included in the diet.

FBW, BWG, and FC were not significantly different in the CS and CO + PG supplemented groups. This result follows Shaw et al. [40], who clarified that increasing the level of CS supplementation in pigs' diets from 0 to 15 % had no significant effect on growth rate. Also, Hemid et al. [41] reported insignificant changes in FBW and BWG in rabbits supplemented with 2.5, 5, and 10 % CS meanwhile, growing rabbits supplemented with 7.5 % CS showed a significant increase in both parameters.

Only CO was able to reduce feed intake when included in the rabbit diet at 1.5 %. Meanwhile, feed intake did not significantly change in the other supplemented groups. These findings may be attributed to the increasing levels of CO [41,42]. Our data consistent with Payvastagan et al. [43] who

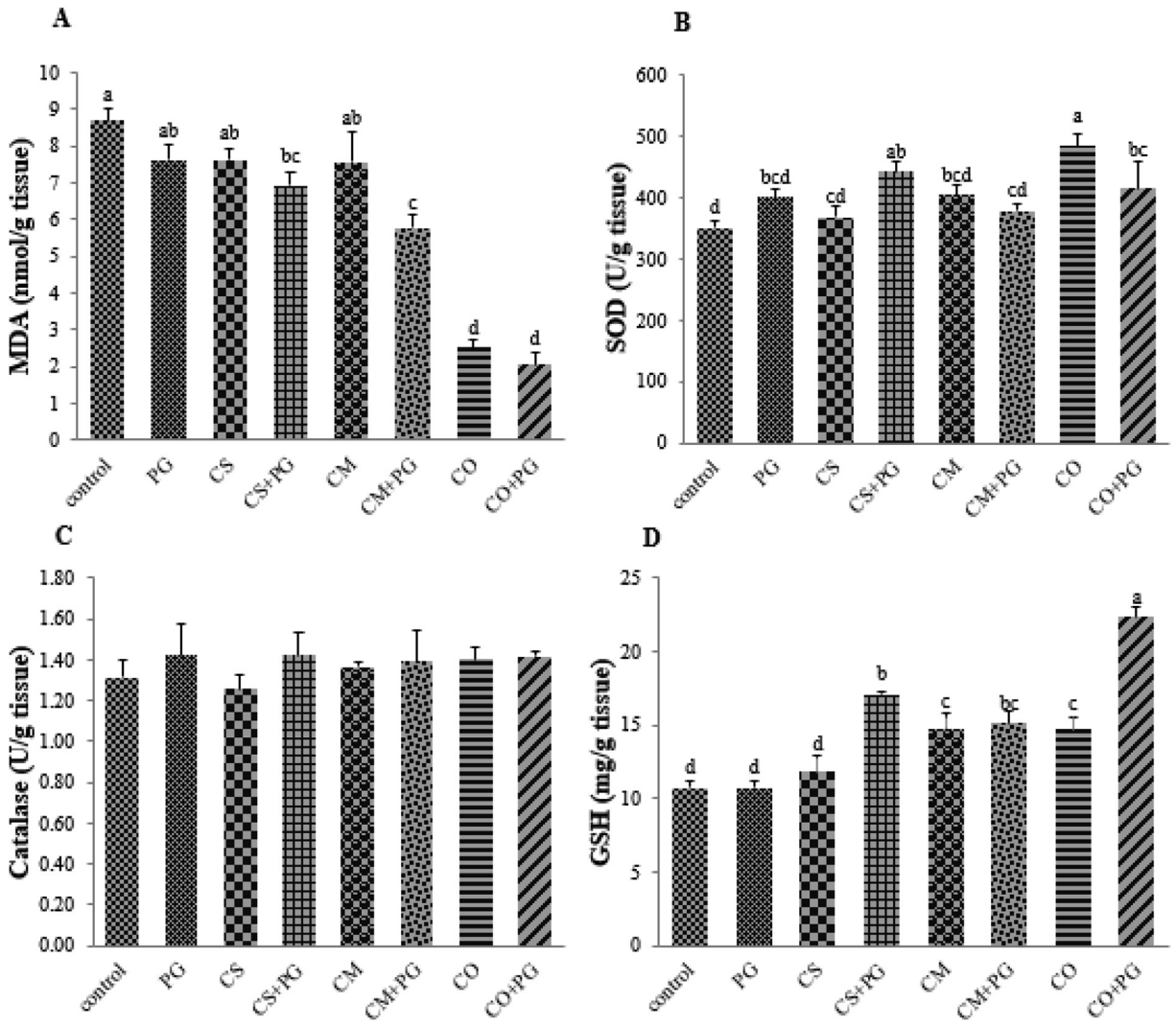

Fig. 2. The hepatic (a) Malondialdehyde level, (b) Superoxide dismutase activity, (c) Catalase activity, (d) Glutathione level in California rabbits at eighth week postdietary supplementation with canola seed (CS), canola meal (CM), canola oil (CO) and/or probiotic (PG).

revealed insignificant changes in feed consumption in young broilers supplemented with 10 and 20 % CM for 21-day. In contrast, Ibrahim et al. [34] recorded a significant increase in FI in rabbits supplemented with different concentrations of CM.

Serum ALT and AST activities, as well as TP, albumin, and globulin levels were not significantly changed in rabbits supplemented with PG. The same results were recorded by Shareef and Al-Dabbagh [44] by the addition of graded levels of yeast in the diet of chicks for 21 days. Also in weaned piglets supplemented with 0.2 % PG for 35 days [45].

In our study, dietary supplementation with CS, CM, and CO, with or without, PG had no significant effect on serum ALT and AST activities. This could be due to low-erucic acid in CM, making it possible to be introduced in growing rabbits' diet at inclusion rates reach to 12−15 % without affecting liver function [46]. We agree with Payvastagan et al. [43] in broilers supplemented with two levels of CM (10−20 %) for 21-day. Alternatively, Ibrahim et al. [34], and Hemid, Abdel-Azeem [35] recorded a significant increase or decrease in plasma AST and ALT concentrations in rabbits supplemented with 50 % or 100 % CM as replacement for SBM, respectively.

In the present study, there was a significant increase in the TP due to increasing globulin levels in CS-, CM-, CM + PG-, CO-, and CO + PG-supplemented rabbits. These findings are in agreement with those of Motlagh [47]. The increase in the TP and globulin may be due to canola being considered an omega-3 resource. Moreover, the higher level TP indicated the higher digestibility of crude protein in these diets [48]. The increase in globulin level is

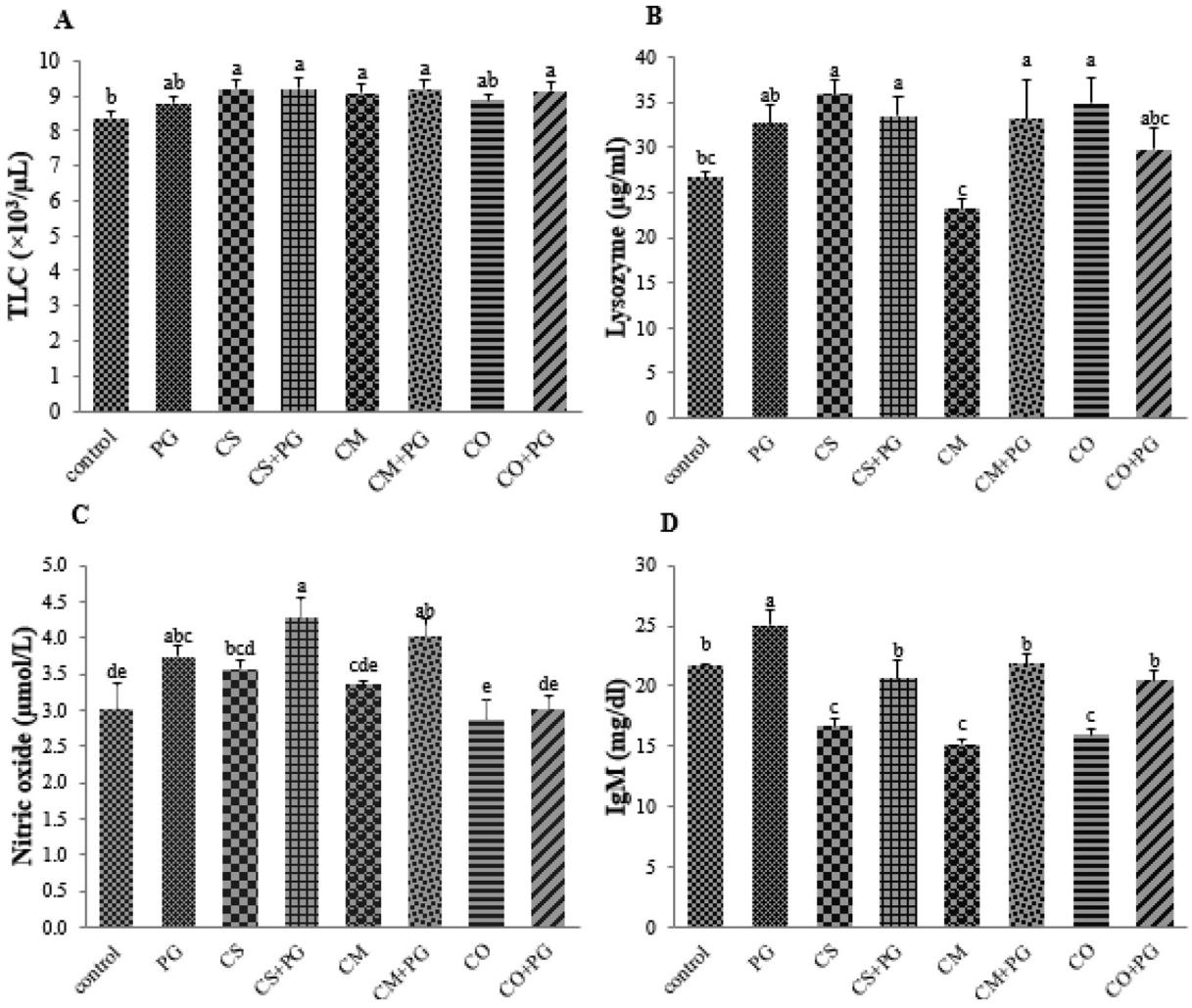

Fig. 3. Immunological parameters (a) TLC, (b) lysozyme activity, (c) nitric oxide level, (D) IgM level in California rabbits at eighth week postdietary supplementation canola seed (CS), canola meal (CM), canola oil (CO) and/or probiotic (PG).

considered a good indicator of increases in immunoglobulin and enhances immunity status [49].

Renal function biomarkers (creatinine and urea) in all the investigated groups did not change significantly. This may be due to the lower content of glucosinolates and erucic acid in canola than in rapeseed [23]. These results are consistent with those reported by Ibrahim et al. [34]. The same result was recorded in piglets supplemented with PG [45]. This was due to the equilibrated nutrient profile of probiotics and their antioxidant activities [50,51]. Ayyat et al. [32] clarified that BUN and creatinine significantly decreased in rabbits supplemented with probiotics for 8 weeks. This was due to the equilibrated nutrient profile of probiotics and their antioxidant activities [52].

Referring to our data, the supplementation of PG induced a significant reduction in the cholesterol level of growing California rabbits, which was attributed to reduced absorption and/or synthesis of cholesterol in the gastrointestinal tract by probiotic supplementation [53,54] or inhibited hydroxymethyl-glutaryl-coenzyme, which is involved in cholesterol synthesis [49]. Abdulrahim et al. [55] demonstrated that probiotic microorganisms reduce cholesterol by preventing bile salts from acting as precursors in their synthesis by deconjugating them in the intestine. This complies with Abdelhady and El-Abasy [49], Ghoneim et al. [56], and Fathi et al. [30], who determined a significant decrease in rabbit serum cholesterol supplemented probiotic.

The serum cholesterol in this study was not significantly affected by CS, CM alone, or in combination with PG and CO + PG. This is in accordance with Ibrahim et al. [34], who found that total lipid were not significantly affected in rabbits supplemented with CM at different doses (6.25, 12.5, 18.75, and 25 %) for 9 weeks. In contrast, a

significant increase in triglyceride levels was recorded in the aforementioned groups. Whilst, Ibrahim et al. [34] and Hemid et al. [35] showed insignificant changes or a significant reduction in total lipid in rabbits supplemented with 6.25−25 % CM for 9 weeks or 100 % CM for 4 weeks, respectively. This may have been due to the higher dose used in our study. Additionally, cholesterol and triglyceride levels were not significantly changed in the CS + PG group compared with those in the control group, which may be attributed to the ameliorative effect of probiotic supplementation, as it may reduce the absorption and/or synthesis of cholesterol in the gastrointestinal tract [51,52].

In our study serum, cholesterol and triglyceride levels were significantly elevated in rabbits supplemented with 1.5 % CO. However, the HDL levels did not change significantly. Our data disagree with those of El-Medany et al. [36] who showed that CO significantly decreased cholesterol in rabbits supplemented with 10 g CO/kg diet for 6 weeks. This difference may be attributed to the higher doses used in our experiments.

The current study illustrated that PG supplementation in growing rabbits did not cause any change in the hepatic MDA, SOD, catalase, and GSH levels. Our findings are in accordance with those of Ghoneim and Moselhy [56]. Other reports have shown that probiotics can decrease MDA when their levels are elevated in harmful conditions, such as a high-fat diet [57] and diabetes [58]. Our results disagree with Chen Gong [57] who observed a significant decrease in SOD in rats on a high-fat diet, even when supplemented with 2 % probiotics for 6 weeks.

The current data showed that hepatic MDA level was significantly decreased in the CM + PG, CO, and CO + PG-supplemented groups. This may be due to the antioxidant activity of canola protein, which makes it convenient for use as a bioactive ingredient in the formulation of functional foods against oxidative stress [59]. This is consistent with Turpeinen et al. [60], who revealed that MDA was significantly decreased when humans consumed 50 g CO/day for 6 weeks. In contrast, Mutalib and Wahle [61] showed that rapeseed oil supplementation in human for 8-weeks did not caused any significant changes in MDA levels.

SOD activity significantly increased in the CS + PG-, CO-, and CO + PG-supplemented groups. In addition, GSH level was significantly increased in all supplemented groups except PG- and CS-supplemented groups it was insignificantly varied, and the highest increase was recorded in the CO + PG supplemented group. The increase in both SOD and GSH may be because canola contains phenolic compounds, which implies their antioxidative power [62].

In the present study, the TLC of rabbits supplemented with PG did not vary significantly. This may be correlated with the balanced nutrient profile of probiotics [50,63]. Our results are in line with those of [64] in mice orally supplemented with lactic acid bacteria. Moreover, Çetïn et al. [21] found that the TLC was not significantly affected by dietary probiotic supplementation at a dose of 1 g/kg for 15 week. Our findings disagreed with those of Ezema and Eze [31], Fathi et al. [30], and Ayyat et al. [32], who demonstrated a significant increase in TLC in rabbit fed a diet containing different concenterations of probiotic.

The inclusion of 15 % CS and CM alone or combined with PG and 1.5 % CO with PG in rabbits' diet showed a significant increase in the TLC. While, El-Medany and El-Reffaei [65] found insignificant changes in TLC when 5 % and 10 % CM supplemented in weaned rabbits' diet for eight weeks. This difference may be attributed to the higher dose used in our study (15 %) than the other previous studies with a balanced amino acid content, which makes CM recommended to be use in animals requiring high levels of methionine, cysteine, and histidine [14,15,66]. In contrast, canola oil alone did not affect total leukocyte count. This is in line with Higgs et al. [67] who illustrated that TLC was not significantly changed by canola oil (14.9 % and 29.85 %) used in the diet of salmon fish for 12 weeks. Meanwhile, a mixture of CO and PG significantly increased the TLC.

PG supplementation did not affect serum lysozyme activity in growing California rabbits. This agrees with El-Ezabi et al. [68] when a low dose of *Lactobacillus Plantarum* was added to fish for 60 days (10^5 CFU/g). Our results showed a significant increase in serum lysozyme activity in the CS, CS + PG, CM + PG, and CO-supplemented groups. This result parallels that of Güroy et al. [69] who reported a significant change in the lysozyme activity of rainbow trout supplemented with CM for 11 weeks. However, it was not significantly affected in the CM and CO + PG-supplemented groups. We agree with Billiar et al. [70] who suggested that canola oil modulates immune functions.

In our study, NO significantly increased in rabbits supplemented with 1.5 % probiotics, Korhonen et al. [71] recommended that *Lactobacillus rhamnosus* induces low-level NO production in macrophages and human T84 intestinal epithelial cells, and hypothesized that induction of NO synthesis may contribute to some of the beneficial effects of probiotics, which

may be involved in the mechanisms of the immunomodulatory action of Probiotic *L. rhamnosus*. Furthermore, the combination of CM and CS with PG increased serum no levels.

A significant increase in serum IgM levels was observed in rabbits supplemented with PG. The beneficial effects of probiotics on immunity may be explained by several unknown mechanisms, but may be explained by probiotics enhancing the proliferation of B lymphocytes in the blood, which may lead to increased serum IgM levels [21]. In addition, probiotics stimulate macrophage activity and activate B-lymphocyte development after they are transported to payer patches through M cells [72]. IgM levels significantly decreased in the CS-, CM-, and CO-supplemented groups. The combination of the aforementioned groups and PG showed an insignificant change in IgM. We referred to the PG antagonist as the decreasing effect of canola on IgM.

5. Conclusion

Our results indicate that probiotics and canola varieties are safe feed additives. Based on the health impacts of various forms of canola used in growing rabbit diets (seed, meal, and oil), we found that CO supplementation had the most beneficial effect on rabbits, followed by CM and CS. The combination of CS with probiotics revealed the most prominent positive results on rabbit health without adverse effects on lipogram, followed by the combination of probiotic with CM and a combination of probiotics with CO.

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Author contributions

MA; methodology, formal analysis, data curation, writing original draft, review, and editing. HA, ER, and FA; conceptualization, validation, visualization, editing final draft and supervision. FA; final reviewing and preparing the manuscript for publication. All authors read and approved the final manuscript.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and available on request from the corresponding author.

Conflicts of interest

There is no conflict of interest in the current research work.

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