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THE USE OF MORINGA LEAVES EXTRACT IN RABBIT DIETS: ITS EFFECT ON PERFORMANCE, LIPID PROFILE, KIDNEY AND LIVER FUNCTION, IMMUNITY, ANTIOXIDANT, DIGESTIVE ENZYMES, AND CECAL MICROBIOTA

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Abstract

This study evaluated the role of *Moringa oleifera* leaves extract (MOLEx) in improving the performance and health of rabbits during the fattening period. The growth, carcass measurements, serum immunity, lipid profile, liver and kidney functions, digestive enzymes, antioxidant condition, cecal microbiota of rabbits have been examined. A total of 100 New Zealand White male rabbits (5-week-old) were randomly distributed into five groups and were fed on the basal diet only or the basal diet supplemented with graded levels of MOLEx (1, 2, 3 or 4 g/kg diet) for 8 weeks. Animals in each group were divided into 10 replicates, with two animals each. Results showed that dietary supplementation of MOLEx at levels of 1, 3 and 4 g/kg feed improved growth performance (P<0.05) including LBW (7, 9, 11, 13 weeks), BWG (5–13 weeks) and FCR (11–13 and 5–13 weeks). The inclusion of MOLEx in rabbits' feed (3 and 4 g/kg) increased carcass %, dressing % and spleen % and decreased duodenum pH and cecal pH. Abdominal fat % was significantly lowered after the treatment with MOLEx. Lipid profile, liver and kidney functions, and cecal microbiota (increasing beneficial bacteria and reducing harmful bacteria) of growing rabbits were positively affected by MOLEx levels. The dietary supplementation of MOLEx improved most of antioxidant biomarkers of growing rabbits. The dietary supplementation of MOLEx (2, 3 and 4 g/kg) increased immunological indicators (IgM, IgG, IgA, lysozyme and complement 3), and lowered cortisol level compared with the control group. Digestive enzymes (protease, lipase and amylase) activities of rabbits were positively affected by MOLEx levels (3 and 4 g/kg feed). In conclusion, the inclusion of MOLEx in rabbits feed can be effective in improving productive performance, kidney and liver functions, digestive enzymes, antioxidant biomarkers, immunological indicators, cecal microbiota and carcass traits.

Key words: *Moringa oleifera*, production, blood metabolites, digestive enzymes, rabbit

Rabbit production plays an important role in meeting the growing demand of meat and achieving global food security. Excellent meat from rabbits is available for human use, and they could be a major solution to some of the meat shortage issues. The use of antibiotic products as growth promoters in animal feed has been banned owing to increasing worries about antibiotic-resistant bacterial strains and the presence of residues in meat and their transmission from animal to human (Saeed et al., 2021; Alagawany et al., 2022). Animal scientists and veterinarians are now turning attention to natural and safe alternatives to replace antibiotics. The biological and physiological effects of the natural components (from medicinal plants) are preferable than those of chemical components (Arif et al., 2022; Bilal et al., 2022). Plant-derived products are favored as feed supplements due to their high antioxidant capacity, low toxicity, and environmentally benign nature while treating a variety of illnesses (Abd El-Hack et al., 2018; Mutwedu et al., 2022). By neutralizing free radicals before they can assault the cell and preventing damage to lipids, proteins, enzymes, carbohydrates, and DNA, plant-derived antioxidants can significantly minimize the damage caused by oxidants (Naskar et al., 2010). Additionally, the antibacterial qualities of plant extracts make them viable substitutes for antibiotics in the manipulation of microbial activity in the gastrointestinal tract (Rafeeq et al., 2023). Many studies have demonstrated the benefits of adding plants high in polyphenols to the rabbit's diet for improving growth and digestion rate (Alagawany et al., 2018; Elawn et al., 2020). Consequently, creating a healthier breeding system through the use of phytogenic materials in rabbit feed is a practical and sustainable solution (Dal Bosco et al., 2012). *Moringa oleifera* (MO) is one of the natural feed supplements.

Moringa oleifera has been a focus of attention for researchers around the world. *Moringa oleifera* leaves extract (MOLEx) contains large number of natural antioxidant molecules like flavonoids (kaempferol, quercetin and rutin), phenols (ellagic, gallic, ferulic, and chlorogenic acid), vitamins (C, A, and α-tocopherol), and carotenoids (Siddhuraju and Becker, 2003; Mbikay, 2012; Ma

et al., 2020; Safwat et al., 2024). Moreover, the phytochemical screening of MOLEx elucidated the presence of saponins, alkaloids, steroids, and catecholic substances, signifying the presence of pharmacologically properties (Divi et al., 2012). The main chemical substances in MOLEx were ascorbic acid, α-linolenic acid, diethyl phthalate and glycerine (Hashem et al., 2019). Calcium, copper, iron, manganese, potassium, zinc and magnesium are among the minerals found in MOLEx (Mahmood et al., 2010). The combination of these compounds is essential for growth and improving antioxidant system, antimicrobial properties and reducing disease infestation in the gastrointestinal tract. Other helpful therapeutic properties of the *Moringa oleifera* plant include antioxidant, antispasmodic, antibacterial, hepatoprotective, antihypertensive and antifungal activities (Amaglo et al., 2010; Mbikay, 2012).

Based on some previous investigations, MOLEx contains a number of phytogenic bioactive components with immunomodulatory, antimicrobial and antioxidant activities that can enhance animals' health and productive performance. Khan et al. (2022) demonstrated that providing broiler chicks with MOLEx in drinking water boosted carcass yield, blood metabolites and immunity. MOLEx acts as antioxidant, growth promoter and antibacterial, as well as displays certain affirmative effects on biochemical parameters and immunity (Allam et al., 2016). Additionally, the presence of polyunsaturated fatty acids in MOLEx such as linolenic acid, improves the gut microbiota metabolism and immunity, boosting the digestive system function (Laparra and Sanz, 2010).

It was hypothesized that adding MOLEx in the diets of growing rabbits can induce their productive performance as well as physiological and health status. The current study targeted the growing rabbits to study the influence of MOLEx on various attributes such as productive performance, lipid profile, kidney function, liver function, immunoglobulins, antioxidant condition, digestive enzymes, and cecal microbiota.

Material and methods

Preparation of *Moringa oleifera* leaf extract

Moringa oleifera leaves (MOL) were purchased from the local market, Sharkia governorate, Egypt. The method given in the study of Namadina et al. (2013) was used to make the ethanolic extract of MOL. Moringa leaves were washed well using distilled water (DW) and then allowed to dry for 21 days at room temperature (30–34°C), after that, the dried leaves were milled through a 0.25 mm screen to obtain a fine powder. Next, each one kg dried leaves powder was extracted in one liter ethanol (70%) for two days and then filtered twice using a filter paper with a pore size of 2 µm. The ethanolic extract was condensed by means of a rotary evaporator at 50°C. The obtained MOL ethanolic extract was resuspended (1 g extract in 10 mL DW) and was saved in the refrigerator at 4°C.

Animals, experimental design and diets

A total of 100 New Zealand White male rabbits (5 weeks old) were randomly distributed into five groups and were fed on the basal diet only or on the basal diet supplemented with graded levels of MOLEx (1, 2, 3 or 4 g/kg diet) for 8 weeks. Animals in each group were divided into 10 replicates, with two animals each. The dimensions of the cage were 35 × 40 × 60 cm, and it was equipped with an auto nipple waterer and manual galvanized metal feeder to ensure a constant supply of fresh and clean water and feed. Rabbits were raised under similar conditions in terms of management, environment and hygiene. Prior to the commencement of the experiment, a week was given to rabbits for adaptation. During this time, they were fed a basal diet devoid of MOLEx. The ingredients and chemical composition of the basal diet are demonstrated in Table 1. Nutrient requirements of growing rabbits were completed in the basal diet based on the study of De Blas and Mateos (2010). The ethics statement was in agreement with the guidelines of the Ethics Committee of the Egyptian Research for the Use and Care of Laboratory Animals by Zagazig University (ZU-IACUC/2/F/313/2023).

Table 1. Ingredients and composition of the basal diet

Components	(g/kg feed)	Nutrient levels[2]	(g/kg feed)
Lucerne meal	340	Dry matter	910.5
Soybean meal	140	Crude protein (N × 6.25)	170.9
Barley grain	170	Ether extract	29.8
Wheat bran	220	Ash	86.3
Yellow com	80	Neutral detergent fiber	344.9
Cane molasses	30	Acid detergent fiber	176.5
Dicalcium phosphate	10	Digestible energy (MJ/kg)	10.30
Sodium chloride	5		
DL-methionine	1		
Premix[1]	4		
Total	1000		

[1]Premix contains per kg (minerals and vitamins mixture): vitamin A, 20,000 U; vitamin D_3, 15,000 U; vitamin E, 8.33 g; vitamin K, 0.33 g; vitamin B_1, 0.33; vitamin B_2, 1.0 g; vitamin B_6, 0.33 g; vitamin B_5, 8.33 g; vitamin B_{12}, 1.7 mg; pantothenic acid, 3.33 g; biotin, 33 mg; folic acid, 0.83 g; choline chloride, 200; manganese 80 g; zinc 60 g; iron 30 g; copper 4 g; iodine 0.5 g; selenium 0.1 g; and cobalt 0.1 g.

[2]Determined according to AOAC (2006).

Performance measurements

The trial was continued for 8 weeks (5–13 weeks of age). The rabbits were individually weighed at 5, 7, 9, 11 and 13 weeks of age. Feed intake (FI) of growing rabbits was documented at the end of each period. Accordingly, body weight gain (BWG), FI, and feed conversion ratio (FCR) were calculated.

Blood sampling and analysis

Five rabbits from each group were selected for blood samples collection when they were 13 weeks old. Without injuring the animals, blood samples were taken from the lateral ear vein into a sterile tube. The serum was separated from the blood tubes by centrifuging them at 5000 rpm for approximately 10 minutes. The serum was stored at –20°C until biochemical analysis. The serum content of albumin (g/dL), total protein (TP, g/dL), uric acid (mg/dL), urea (mg/dL), creatinine (mg/dL), triglycerides (mg/dL), total cholesterol (TC, mg/dL), low-density lipoprotein (LDL, mg/dL), high-density lipoprotein (HDL, mg/dL), and very low-density lipoprotein (VLDL, mg/dL), as well as the activity of AST and ALT enzymes (IU/L) were determined using commercial kits (Diamond Diagnostic Company, Giza, Egypt), according to the instructions delivered by the manufacturer. The serum globulin was calculated (globulin = TP – albumin). Serum antioxidant biomarkers were determined by methods designated by: Paglia and Valentine (1967) for glutathione peroxidase (GSH-PX, U/mL), Koracevic et al. (2001) for total antioxidant capacity (TAC, mmol/L), Nishikimi et al. (1972) for superoxide dismutase (SOD, U/mL), Trotti et al. (2002) for reactive oxygen species (ROS), Mueller et al. (1997) for catalase (CAT, U/mL) and Ohkawa et al. (1979) for malondialdehyde (MDA, nmol/mL). Serum concentrations of lysozyme activity (ng/mL), complement component 3 (mg/dL) and immunoglobulins (IgA, IgG and IgM; mg/mL) were measured by using commercial ELISA kits according to the instructions delivered by the manufacturers.

Carcass traits and intestinal pH

At 13 weeks of age, five rabbits from each experimental group were selected and sacrificed at the end of the feeding trial to assess the carcass indices. After complete bleeding, skin, tail and viscera were removed, and then carcass yield and all related parameters were evaluated in accordance with Blasco et al. (1993) procedure. The weights of carcass, liver, kidney, heart, lungs, abdominal fat and spleen, were recorded and stated as % of live pre-slaughter weight. The dressing % and giblets % were calculated. A pH meter (Model 507; Crison Instruments S.A., Barcelona, Spain) was used to measure the pH of the cecal and duodenum content.

Digestive enzyme activities

Small intestine digesta samples were immediately obtained from slaughtered rabbits. Each sample was weighed and diluted ten times using phosphate-buffered saline (PBS, pH=7). Samples were homogenized by using a hand-held glass homogenizer, and then centrifuged for 20 minutes at 5000 rpm (temperature: 4°C). The supernatants were carefully separated after centrifugation and kept at 4°C until analysis. Following the extraction process, the assay of digestive enzymes was performed within a 24-hour period using the techniques outlined by: Lowry et al. (1951) for protease, Boutwell (1962) for lipase and Coles (1986) for amylase.

Cecal microbiota assay

Immediately after the rabbits were slaughtered, 1 g of cecum content from each rabbit was collected and put into Erlenmeyer flasks (250 mL), which contained a sterile solution of peptone saline (0.85% NaCl and 0.1% peptone). The contents were then mixed extensively to obtain a series of dilutions up to 10^{-7}. The counting of *Lactobacillus* was performed by employing the MRS agar, as described by Argyri et al. (2013). Plates of eosin-methylene blue agar were used to enumerate *E. coli*, following performing an incubation at 37°C for a duration of 24 hours, as described by Szabo et al. (1986). Salmonella-Shigella agar (SS agar) was used for enumerating *Salmonella* sp., in accordance with Edwards and Hilderbrand (1976). For enumerating total coliforms, plates of violet red bile agar (VRBA) were used following a 24-hour incubation period at a temperature of 37°C, according to Harrigan and McCance (1976).

Statistical analysis

The one-way ANOVA test was used to do the statistical analysis of data by using the following statistical model:

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

Where: Y_{ij} signifies the observation, G_i signifies the treatment effect (T1:T5), μ signifies the overall mean, and e_{ij} signifies the experimental error. Differences among means were tested by Tukey's test. The level of $P<0.05$ was used as the criterion for statistical significance. Statistical tests were conducted by using the computer software of SPSS program version 17 under Windows operating system (SPSS, 2008).

Results

Growth performance

The impacts of different treatments on the growth performance of growing rabbits are detailed in Table 2. LBW was significantly augmented ($P<0.05$) as affected by dietary MOLEx levels compared with the control group. Adding MOLEx to fattening rabbit diets increased LBW at all ages except at 5 weeks of age relative to that of the control group. The body weight gain during 5–7 and 5–13 weeks of age of rabbits fed MOLEx-supplemented diets (3 and 4 g/kg feed) were higher than the control rabbits. The group fed 1 g MOLEx/kg diet was significantly higher than the control only during the whole period 5–13 weeks of age. However, no significant difference in BWG was found between control and others groups during the phases 7–9, 9–11 and 11–13 weeks of age. Feed intake was not affected by treatments; conversely, it was lower ($P = 0.0098$) in the MOLEx groups during the last phase (from 11 to 13 weeks of age) than in the control group except T4 (3 g MOLEx/kg feed). Values of FCR did not differ among the experimental groups during the first periods (5–7, 7–9 and 9–11 weeks of age). During 11–13 weeks of age, FCR

was significantly bettered (P=0.0445) in rabbits fed 1, 3 and 4 g MOLEx/kg feed compared to those fed the control diet. In the whole period (7–13 weeks of age), all levels of MOLEx improved FCR values.

Carcass traits and intestinal pH

Data presented in Table 3 show the impacts of different MOLEx levels on carcass traits and intestinal pH of growing rabbits. Carcass %, dressing % and spleen % were significantly increased in rabbits treated with MOLEx at levels of 3 and 4 g/kg feed compared to the control rabbits. Rabbits fed on diets treated with different levels of MOLEx (1, 2, 3 and 4 g/kg feed) displayed significantly lower levels of abdominal fat % compared to the control group. However, no differences were caused by MOLEx in liver %, heart %, kidney %, giblets % and lungs %. Duodenum pH and cecal pH were significantly lowered (P<0.01) after the inclusion of MOLEx (3 and 4 g/kg feed) in rabbits' feed compared with the control diet.

Liver and kidney functions

Table 4 shows the impacts of dietary MOLEx addition on kidney and liver functions of growing rabbits. The rabbits fed diets containing different MOLEx levels had higher serum total protein and globulin than those in the control group. Adding MOLEx in rabbit diets significantly lowered AST (levels: 3 and 4 g/kg diet; P=0.0277), ALT (levels: 1, 2 and 3 g/kg diet; P=0.0001) and creatinine (all MOLEx levels; P=0.0001) compared to the control group. Albumin, albumin/globulin ratio, urea and uric acid values were not affected by MOLEx levels (P>0.05).

Lipid profile

Lipid profile results are summarized in Table 5. The inclusion of different MOLEx levels in rabbit feed decreased values of total cholesterol (P=0.0052), triglycerides (P=0.0005), LDL (P=0.0032) and VLDL (P=0.0005) compared to the control group. Higher HDL level was noted in the rabbits belonging to the MOLEx group (3 g/kg diet) compared to the control group (P=0.0052).

Immunity indicators and cortisol

Data revealed in Table 6 show the effects of MOLEx levels on immunological indicators and cortisol level of growing rabbits. The rabbits fed diets containing MOLEx (1, 2, 3 and 4 g/kg feed) had significantly higher IgM, IgA and lysozyme compared with that in the control group. Complement 3 and IgG values were increased in rabbits fed diets supplemented with MOLEx (2, 3 and 4 g/kg feed) compared with the control group. Regarding blood cortisol levels, adding MOLEx into the diet of growing rabbits (1, 2, 3 and 4 g MOLEx/kg feed) significantly decreased (P<0.0001) their cortisol levels by 27.1, 33.0, 43.3 and 41.4%, respectively, compared to those fed the control diet.

Table 2. Growth performance of growing rabbits as affected by different levels of dietary *Moringa oleifera* leaves extract

Items	*Moringa oleifera* leaves extract levels (g/kg diet)					SEM	P value
	0	1	2	3	4		
Live body weight (g) at							
5 weeks	621.0	622.7	621.0	617.5	619.5	8.227	0.9932
7 weeks	1079 b	1132 a	1112 ab	1139 a	1164 a	15.358	0.0304
9 weeks	1464 c	1551 b	1497 c	1584 ab	1612 a	18.339	0.0005
11 weeks	1733 c	1875 b	1784 c	1900 ab	1950 a	19.791	<.0001
final (13 weeks)	1893 c	2075 b	1930 c	2107 ab	2156 a	16.964	<.0001
Body weight gain (g/day)							
5–7 weeks	32.75 b	36.38 ab	35.11 ab	37.25 a	38.89 a	1.087	0.0403
7–9 weeks	27.46	29.93	27.46	31.79	32.04	2.028	0.3813
9–11 weeks	19.24	23.14	20.50	22.57	24.11	1.424	0.2064
11–13 weeks	11.44	14.29	10.44	14.82	14.71	1.137	0.0818
overall (5–13 weeks)	22.72 c	25.93 b	23.38 c	26.61 ab	27.44 a	0.250	<.0001
Feed intake (g/day)							
5–7 weeks	73.00	72.50	71.50	68.00	71.00	2.309	0.6070
7–9 weeks	75.50	72.50	69.50	70.00	74.00	2.598	0.5122
9–11 weeks	66.00	68.50	62.00	65.33	64.33	2.996	0.6603
11–13 weeks	73.00 a	58.50 bc	51.50 c	63.00 ab	58.50 bc	3.175	0.0098
overall (5–13 weeks)	71.88	68.00	63.63	66.58	66.96	1.459	0.0531
Feed conversion ratio (g feed/g gain)							
5–7 weeks	2.23	2.00	2.04	1.84	1.83	0.096	0.0956
7–9 weeks	2.77	2.43	2.56	2.23	2.31	0.130	0.1218
9–11 weeks	3.43	2.98	3.05	2.91	2.69	0.177	0.1338
11–13 weeks	6.53 a	4.16 b	4.97 ab	4.28 b	4.09 b	0.485	0.0445
overall (5–13 weeks)	3.17 a	2.62 bc	2.72 b	2.50 bc	2.44 c	0.070	0.0004

SEM stands for standard error of the mean.
Means in a row with different letters differ significantly (P<0.05).

Table 3. Carcass traits and intestinal pH of growing rabbits as affected by different levels of dietary *Moringa oleifera* leaves extract

Items	*Moringa oleifera* leaves extract levels (g/kg diet)					SEM	P value
	0	1	2	3	4		
Carcass (%)	54.05 c	55.22 bc	55.09 bc	56.03 ab	57.34 a	0.429	0.0040
Liver (%)	3.33	3.47	3.32	3.45	3.60	0.142	0.6587
Kidney (%)	0.45	0.46	0.44	0.47	0.47	0.037	0.9771
Heart (%)	0.29	0.35	0.30	0.32	0.35	0.018	0.2135
Lungs (%)	0.66	0.73	0.69	0.72	0.75	0.099	0.9693
Abdominal fat (%)	1.29 a	1.06 b	0.91 bc	0.92 bc	0.82 c	0.058	0.0022
Spleen (%)	0.09 c	0.11 c	0.11 bc	0.13 ab	0.14 a	0.006	0.0022
Giblets (%)	4.07	4.28	4.06	4.23	4.42	0.151	0.4853
Dressing (%)	58.12 c	59.50 bc	59.15 bc	60.27 b	61.76 a	0.437	0.0027
Duodenum pH	6.81 ab	6.79 ab	6.84 a	6.74 bc	6.67 c	0.022	0.0033
Cecal pH	7.18 a	7.16 a	7.11 ab	7.01 bc	6.96 c	0.035	0.0071

SEM stands for standard error of the mean.
Means in a row with different letters differ significantly (P<0.05).

Table 4. The liver and kidney functions of growing rabbits as affected by different levels of dietary *Moringa oleifera* leaves extract

Items	*Moringa oleifera* leaves extract levels (g/kg diet)					SEM	P value
	0	1	2	3	4		
TP (g/dL)	5.13 c	5.63 b	5.57 b	6.04 a	6.26 a	0.085	<.0001
ALB (g/dL)	2.81	2.83	2.85	2.95	2.91	0.121	0.9341
GLOB (g/dL)	2.33 d	2.80 bc	2.73 c	3.10 ab	3.35 a	0.097	0.0005
A/G (%)	1.21	1.02	1.05	0.96	0.87	0.072	0.1185
AST (IU/L)	19.92 a	14.44 ab	15.47 ab	10.17 b	12.14 b	1.748	0.0277
ALT (IU/L)	61.15 a	47.15 b	45.87 b	44.38 b	67.18 a	2.267	0.0001
Creatinine (mg/dL)	1.11 a	0.55b	0.44 b	0.50 b	0.54 b	0.059	0.0001
Urea (mg/dL)	40.61	36.08	36.94	37.96	37.31	1.183	0.1649
Uric acid (mg/dL)	0.23	0.20	0.21	0.22	0.23	0.025	0.8517

SEM stands for standard error of the mean.
Means in a row with different letters differ significantly (P<0.05).
TP: total protein, ALB: albumin, GLOB: globulin, A/G ratio: albumin/globulin ratio; AST: aspartate transaminase; ALT: alanine transaminase.

Table 5. Lipid profile of growing rabbits as affected by different levels of dietary *Moringa oleifera* leaves extract

Items	*Moringa oleifera* leaves extract levels (g/kg diet)					SEM	P value
	0	1	2	3	4		
TC (mg/dL)	136.30 a	122.99 b	108.43 c	120.49 bc	112.49 bc	3.632	0.0052
TG (mg/dL)	215.65 a	183.00 b	138.85 d	168.35 bc	148.05 cd	8.198	0.0005
HDL (mg/dL)	52.07 bc	55.16 bc	48.42 c	66.82 a	58.32 b	2.439	0.0052
LDL (mg/dL)	41.10 a	31.23 b	32.25 b	20.00 c	24.56 bc	2.694	0.0032
VLDL (mg/dL)	43.13 a	36.60 b	27.77 d	33.67 bc	29.61 cd	1.640	0.0005

SEM stands for standard error of the mean.
Means in a row with different letters differ significantly (P<0.05).
TC: total cholesterol; TG: triglycerides; HDL: high density lipoproteins; LDL: low density lipoproteins; VLDL: very low-density lipoproteins.

Table 6. Immunity indicators and cortisol of growing rabbits as affected by different levels of dietary *Moringa oleifera* leaves extract

Items	*Moringa oleifera* leaves extract levels (g/kg diet)					SEM	P value
	0	1	2	3	4		
IgM (mg/mL)	1.34 c	4.19 a	3.25 b	3.35 b	2.75 b	0.250	0.0002
IgG (mg/mL)	1.81 b	2.63 ab	3.12 a	3.05 a	3.48 a	0.256	0.0153
IgA (mg/mL)	0.16 c	0.38 a	0.29 b	0.31 b	0.40 a	0.025	0.0006
Lysozyme (ng/ml)	2.34 b	5.75 a	6.41 a	5.21 a	6.30 a	0.512	0.0015
Complement 3 (mg/dL)	16.44 c	24.20 cb	33.91 b	31.74 b	46.51 a	2.977	0.0004
Cortisol (mg/dL)	2.03 a	1.48 b	1.36 bc	1.15 d	1.19 cd	0.050	<.0001

SEM stands for standard error of the mean.
Means in a row with different letters differ significantly (P<0.05).
IgM: immunoglobulin M; IgG: immunoglobulin G; IgA: immunoglobulin A.

Table 7. Biomarkers of oxidant-antioxidant status of growing rabbits as affected by different levels of dietary *Moringa oleifera* leaves extract

Items	*Moringa oleifera* leaves extract levels (g/kg diet)					SEM	P value
	0	1	2	3	4		
SOD (U/mL)	61.60 d	158.00 a	121.50 b	113.50 bc	96.00 c	6.305	<.0001
MDA (nmol/mL)	4.79 a	1.52 b	1.39 b	1.78 b	1.45 b	0.231	<.0001
TAC (ng/ml)	1.62 c	5.13 a	2.66 bc	3.01 bc	3.80 ab	0.464	0.0069
CAT (ng/ml)	40.43 c	53.14 b	49.81 b	62.59 a	40.64 c	2.615	0.0007
GPX (U/mL)	198.00 c	278.50 a	233.50 b	250.50 ab	269.50 a	8.776	0.0006
ROS (U/ml)	0.18 c	0.42 ab	0.23 bc	0.43 ab	0.62 a	0.061	0.0086

SEM stands for the standard error of the mean.
Means in a row with different letters differ significantly (P<0.05).
SOD: superoxide dismutase; MDA: malondialdehyde; TAC: total antioxidant capacity; CAT: catalase; GPX: glutathione peroxidase; ROS: reactive oxygen species.

Table 8. Digestive enzymes' activity of growing rabbits as affected by different levels of dietary *Moringa oleifera* leaves extract

Items	*Moringa oleifera* leaves levels (g/kg diet)					SEM	P value
	0	1	2	3	4		
Amylase (U/L)	25.31 c	32.66 bc	37.60 b	65.95 a	68.94 a	2.263	<.0001
Lipase (U/L)	34.00 c	51.25 bc	57.00 b	148.00 a	135.25 a	4.965	<.0001
Protease (IU/L)	108.00 c	158.20 a	103.70 c	132.71 b	152.60 ab	7.425	0.0012

SEM stands for the standard error of the mean.
Means in a row with different letters differ significantly (P<0.05).

Table 9. Cecal microbiota (Log CFU/g) of growing rabbits as affected by different levels of dietary *Moringa oleifera* leaves extract

Items	*Moringa oleifera* leaves extract levels (g/kg diet)					SEM	P value
	0	1	2	3	4		
Lactobacillus sp.	8.15 b	9.00 a	9.20 a	9.45 a	9.26 a	0.118	0.0006
E. coli	7.68 a	6.44 b	6.09 bc	5.45 c	4.68 d	0.164	<.0001
Salmonella sp.	5.84 a	4.84 b	3.58 c	3.14 d	3.00 d	0.074	<.0001
Coliform	7.72 a	6.96 b	6.60c	5.80 d	5.07 d	0.097	<.0001

SEM stands for the standard error of the mean.
Means in a row with different letters differ significantly (P<0.05).

Antioxidant biomarkers

Impacts of dietary supplementation of MOLEx on antioxidant biomarkers of rabbits are presented in Table 7. MDA concentration was significantly decreased (P<0.0001) in the groups treated with graded levels of MOLEx compared with the control group. Feeding rabbits with diets containing graded levels of MOLEx (1, 2, 3 and 4 g/kg feed) significantly increased antioxidant biomarkers (SOD and GSH-PX). The obtained results for others indices showed inconsistent figures and did not follow a definite trend regarding the effect of MOLEx levels. We noted that the inclusion of MOLEx in rabbit diets significantly increased TAC (levels: 1 and 4 g/kg feed; P=0.0069), CAT (levels: 1, 2 and 3 g/kg; p=0.0007) and ROS (levels: 1, 3 and 4 g/kg; P=0.0086) compared with the control group.

Digestive enzymes

The influences of dietary inclusion of MOLEx on digestive enzymes of growing rabbits are detailed in Table 8. The inclusion of MOLEx in rabbit diets significantly increased amylase and lipase (levels: 2, 3 and 4 g/kg feed; P<0.0001) and protease (levels: 1, 3 and 4 g/kg; P=0.0012) compared with the control group.

Cecal microbiota

Findings of Table 9 stated that the cecal microbiota of growing rabbits was positively affected by different MOLEx levels. Rabbits fed diets containing graded levels of MOLEx (1, 2, 3 and 4 g/kg diet) had significantly decreased *E. coli*, *Salmonella* and *Coliform* populations number (P<0.001), and increased *Lactobacillus* sp. count (P=0.0006) compared with the control group.

Discussion

The *M. oleifera* extract has nutritional and bioactive components that could increase the productive performance of rabbits. Alabi et al. (2017) indicated that MOLEx can be used as a growth promoter. In the current study, the performance indices of growing rabbits were positively affected by graded levels of MOLEx. This result agreed with that mentioned by Akhouri et

al. (2013) who revealed that MOLEx significantly augmented BWG and improved feed conversion efficiency in poultry. Hashem et al. (2019) illuminated that using MOLEx in rabbit nutrition boosted final BW and FCR compared to the control. El-Kholy et al. (2018) revealed that Moringa leaves extract significantly augmented the final BW, BWG and improved FCR. Falemara et al. (2020) suggested that including Moringa leaves in the experimental diets contributed to improve the growth performance (growth rate, FI and FCR) of rabbits. Additionally, the best FCR, final BW, daily weight gain and performance index were recorded in rabbits fed *Moringa oleifera* leaves (El-Adawy et al., 2020). In the current study, the affirmative influences of MOLEx on the final BW, BWG, and FCR could be associated with some identified chemical components in the extract. Moreover, the biological role of MO as a naturally occurring growth promoter may be responsible for the generated augmentation of growth performance of growing rabbits (El-Badawi et al., 2014). Also, it could be linked to the impact of the available nutritional compounds and some growth stimulating substances of *Moringa oleifera* that lead to improve BW (Kakengi et al., 2007). According to Hashem et al. (2019), MOLEx may directly supply animals with certain nutrients – like vitamins and fatty acids – that are critical for growth. These substances may also function as prebiotics for microbiota, improving fiber digestion (Caceres et al., 1991). El-Badawi et al. (2017) stated that *Moringa oleifera* augmented the count of useful gut microbes, boosting the productive performance of rabbits. Improvement of BW occurred in rabbits fed Moringa might be due to its high antioxidant content (Helal et al., 2017). Kumar et al. (2021) recommended that the enhancement in BW after feeding MOLEx might be owing to various phytochemicals present in MOLEx affecting broad aspect of physiology, for instance, nutrient absorption and bioavailability in animal. Finally, the better growth indices shown in MOLEx-treated rabbits might be because of phytogenic substances and immunomodulatory and antioxidant properties of *Moringa oleifera*.

In the current study, the results showed positive changes in carcass traits, which agreed with those described by El-Kholy et al. (2018) who stated that the inclusion of MOLEx in rabbit diets increased the carcass percentage. The best carcass in MOLEx-treated rabbits may be associated with increasing growth performance. Omara et al. (2018) detected that there were enhancements in the slaughter weight, carcass % and dressing %, but abdominal fat weight was diminished with increasing *Moringa oleifera* levels in the diet. El-Desoky et al. (2018) pointed out that rabbits fed a diet supplemented with *Moringa oleifera* had significantly higher carcass % and dressing %. Nayel (2021) elucidated that the growing rabbits administered with MOLEx had heavier carcass weight compared with the control group. El-Kholy et al. (2018) revealed that the abdominal fat percentage was decreased by the addition of MOLEx. According to Cui et al. (2018), USFA in MOL may increase fatty acid ß-oxidation and thus decrease the accumulation of abdominal fat. El-Badawi et al. (2016) mentioned that the growing rabbit diet supplemented with MOL reduced MDA concentration and increased TAC, which could be linked to a decrease in fat deposition by either increasing the hormone-sensitive lipase activity in the adipose tissue or decreasing the activities of lipoprotein lipase and malate dehydrogenase (Lu et al., 2007). The decrease in the percentage of abdominal fat in rabbits fed MOLEx may be due to the ability of MOLEx to increase beneficial bacteria (as shown in the current study), for instance *Lactobacillus* that decrease acetyl-CoA carboxylase activity (the rate-limiting enzyme in the synthesis of fatty acids) (Toghyani et al., 2011).

Blood components are a suitable indicator of the nutritional state of the animals. Some medicinal plants are mainly used as food supplements, or for medicinal purposes, and thus engage in a series of physiological reactions, which may lead to a change in biochemical parameters in the blood (El-Saadony et al., 2023; Toson et al., 2023). In the current study, positive impacts of dietary MOLEx supplementation on kidney and liver functions of growing rabbits were detected. Similarly, El-Kholy et al. (2018) declared that adding MOLEx to drinking water for growing rabbits had a positive impact on serum biochemical responses without any harmful influences on kidneys and liver functions. Improved immune and liver functioning in the MOL-fed rabbits were indicated by higher serum levels of total protein and globulin and decreased levels of ALT and AST (Selim et al., 2021). This finding is consistent with those of Wallace et al. (2010) and Mbikay (2012) who discovered that some MOLEx constituents had strong immunostimulant properties. The presence of polyphenolic chemicals and flavonoids in MOLEx has been linked to immunological function (Amaglo et al., 2010; Okwari et al., 2013). MOLEx decreased creatinine in renal ischemic rats' models (Akinrinde et al., 2020). Leaves supplementation reduced serum creatinine in chicken (Abdel-Wareth and Lohakare 2021). The reduction in creatinine shows the nephroprotective property of MOLEx on the health of rabbits.

Moreover, MOLEx was stated to have a hepatoprotective activity owing to its content of bioactive flavonoids, for instance quercetin (Mishra et al., 2011; Farooq et al., 2012). The improvements in liver and kidney functions of rabbits fed diets supplemented with MOLEx may be owing to its content of high antioxidant levels that support the body's ability to carry out its activities and preserve the integrity and health of body tissues.

In the current study, the inclusion of different MOLEx levels in rabbit feed improved the lipid profile. This finding is consistent with the study of El-Kholy et al. (2018) who clarified that the rabbits in the MOLEx-treated groups had lower total cholesterol and triglycerides than the rabbits in the control group. Selim et al. (2021) displayed that serum cholesterol, triglycerides and LDL were significantly lessened in the *Moringa oleifera*

groups compared to the control. It has been reported that the improvement of lipid levels in many studies was due to the substances found in Moringa, which act as an effective antioxidant. Ghasi et al. (1999) demonstrated that the juice of Moringa leaves was proven to be a powerful hypocholesterolemic agent, which may be owing to two mechanism actions by decreasing the biosynthesis of cholesterol and absorption of feed cholesterol (Souza et al., 2007). The hypolipidemic activity of numerous therapeutic plants has been related to bioactive substances like alkaloids and saponins. According to Patil et al. (2010), alkaloids may mediate hypolipidemic action either by promoting fecal bile acid excretion or by upregulating the activities of lipolytic enzymes. Saponins are known to impair the digestion and limit the capacity of intestinal mucus to absorb nutrients, especially lipids, which are one of the important sources of energy (Dei et al., 2007). Furthermore, β-sitosterol and quercetin may be key cholesterol-lowering constituents of MOLEx, as they may act singly or in synergy with other bioactive constituents, improving lipid profile and reducing fat deposition (Pop et al., 2018; Zhu and Jiang, 2022).

Enhancing animal productivity is considered largely dependent on the use of nutritional antioxidants (Elwan et al., 2019). In the current study, improvements in the antioxidant biomarkers in growing rabbits treated with MOLEx were observed. Notably, in numerous experimental models, MOLEx exhibited the ability to increase the intracellular antioxidant defense system (CAT, GSH and SOD) and reduced MDA (Mthiyane et al., 2022). Hashem et al. (2019) indicated that the treatment with MOLEx boosted the activity of antioxidant enzymes but decreased the MDA concentration of growing rabbits. The total antioxidant capacity was increased consistently with increased MOLEx concentration in rabbit diets, enhancing the oxidative status of rabbits (Ojo et al., 2017). Polysaccharides isolated from MO enhanced GSH-PX, CAT and SOD activities and reduced ROS and MDA levels (Gu et al., 2022). The presence of significant concentrations of vitamin C, the most powerful antioxidant, as well as other active compounds with antioxidant potential, like isobenzofuran-1-one-3-acetic acid and phytol, may be responsible for MOLEx's antioxidant activity (Kadhim et al., 2016; Ezhilan and Neelamegam, 2012). *Moringa oleifera* has been recognized to possess various antioxidants with high concentrations, for instance glucosinolate and flavonoids like rutin, quercetin and kaempferol, as well as phenolic acids (chlorogenic, ellagic, caffeic, ferulic and gallic acid) (Mbikay, 2012). These antioxidants can prevent oxidative damage via boosting antioxidant enzymes that decrease the creation of lipid peroxidation and free radicals (Sreelatha and Padma, 2009). Furthermore, the antioxidant impact of MOLEx may be owing to the presence of polyphenols, anthocyanin, glycosides and thiocarbamates that eradicate free radicals (Luqman et al., 2012).

In the current study, the dietary supplementation of MOLEx boosted immunological indicators. The presence of phenolic compounds and flavonoids in MOLEx may be factors that contribute to enhance immune responses (Hosseinzade et al., 2019). Also, MOLEx-treated rabbits had significantly augmented lysozyme activity, and immunoglobulin G concentrations (El-Desoky et al., 2022). The beneficial impacts of MOLEx on immune system performance can be attributed to the high concentrations of different fatty acids found in MOLEx. Mahmoud et al. (2022) stated that the strengthening of immune system in animals fed MOLEx may be due to it containing some polyphenols, phenolics, terpenoids and flavonoids that have immune-stimulating functions. Additionally, the improved immunity of animals treated with MOLEx may be due to antioxidant activities of some components of MOLEx like vitamins (C and E) and to the capacity of polysaccharides to modulate the immune system (Teteh et al., 2013).

Medicinal herb feed additives can enhance digestive enzyme activity (Alagawany et al., 2021). In the present study, the inclusion of MOLEx in rabbit diets significantly increased digestive enzymes. Thus, the major active components in MOLEx can boost the digestive enzyme activities and provide favorable influences on nutrient digestibility. Polysaccharides isolated from *Moringa oleifera* can upregulate the secretion of enzymes involved in the absorption and digestion of protein in rabbits (Khalid et al., 2022). The active ingredients in MOLEx improve nutrient metabolism and utilization through increasing pancreatic activity (Siti et al., 2019). MO polysaccharides significantly enhanced protease and amylase activities in goats (Afzal et al., 2021). Moreover, vitamin C and phenolic compounds found in *Moringa oleifera* can protect the cell membranes of the digestive system from oxidative stress via scavenging O_2 radicals, consequently boosting feed utilization (Mutwedu et al., 2022). Moreover, the presence of polyunsaturated fatty acids like α-linolenic acid in MOLEx (n-3 fatty acid) influenced the gut microbiota's metabolism and immunity, inducing gastrointestinal functions (Laparra and Sanz, 2010).

Plant-derived feed supplements have biologically active constituents and antimicrobial activity (Rafeeq et al., 2022; Dhama et al., 2023). In the current study, the cecal microbiota of growing rabbits was positively affected by different MOLEx levels. Thus, MOLEx was used as an alternative to antibiotics and could be a favorable natural antimicrobial agent due to its antibacterial properties. Allam et al. (2016) revealed that MOLEx inhibited the growth of *E. coli* and *Salmonella* species in chickens. Adding MOLEx in drinking water for growing rabbits boosted the microbial ecology of the gastrointestinal tract, decreasing pathogenic bacteria such as *E. coli* and *Clostridium* (El-Kholy et al., 2018). Aljohani and Abduljawad (2018) illustrated that the bacteria count in the cecal content of rabbits fed a diet supplemented with MOL was significantly lower than that in control rabbits. According to Abd El-Hack et al. (2018), there are several phytochemicals found in *Moringa oleifera* that have antibacterial properties. Additionally, El-Desoky et

al. (2021) stated that MOLEx has various active constituents with antimicrobial/anticoccidial activity, including 2-furoic acid, salinomycin, lactacystin, rifabutin and 9S, 11, 15 S-trihydroxythrombox-13E-enoic acids. These components act directly on pathogenic bacteria and lead to inhibition of its growth via disrupting the synthesis of cell membranes or essential enzymes (Kimse et al., 2009). Furthermore, the presence of bioactive compounds in *Moringa oleifera* may be responsible for the antimicrobial effect through their ability in interrupting some metabolic processes and inhibiting the microbial growth (Godstime et al., 2014). *Moringa oleifera* moderates cell turnover, thereby reducing the intestinal competition of available nutrients (Voemesse et al., 2019). It is possible that MOLEx is given as a useful additive in rabbit nutrition because of its positive effects on productive and physiological performance in this study. The results revealed that the inclusion of MOLEx up to 4 g/kg in rabbit feed can be effective in improving growth performance, carcass traits, kidney and liver functions, digestive enzymes, antioxidant biomarkers, immunological indicators, and cecal microbiota, reflecting the good health status of growing rabbits. The recommended level of MOLEx based on the study results is 4 g/kg diet.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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