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Milk thistle seeds and rosemary leaves as rabbit growth promoters

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The trial aimed to study milk thistle seeds (MTS) and rosemary leaves (RL) as natural growth promoters for rabbits during 28-91 days of age. A total of 100 weaned rabbits were distributed into 5 groups (20 rabbits/group) fed the same basal diet. The 1st group (control) was unsupplemented, the 2nd and 3rd groups were supplemented with MTS at 5 and 10 g/kg, while the 4th and 5th groups were fed the basal diet supplemented with RL at 5 and 10 g/kg, respectively. MTS at 10g/kg significantly increased growth rate compared with the same dose of RL. In comparison to the control both MTS and RL5 were found to significantly increase growth rate, while MTS at both levels of supplementation resulted in a significant increase of feed intake. All the supplemented groups had a better feed conversion ratio than the control, with the best values obtained in the MTS10 and RL5 groups. Digestibility of crude protein, organic and dry matter of the MTS10 and RL5 groups exceeded those in the other groups. In relation to the control MTS and RL significantly increased red blood cell counts (RBCs), while RL also increased PCV, Hgb and MCHC. Lymphocyte counts

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were significantly increased at 5g/kg of both supplements compared to the control and the RL10 groups. There were significant decreases in the ALT and ALT/AST ratios in the supplemented groups compared to the control, while total anti-oxidant capacity (TAC) was higher in the supplemented groups. The total and LDL cholesterol levels were the lowest in the RL10 group. The MTS10 group had a higher dressing percentage compared with the RL10 and control groups. In turn, the MTS10 group showed moderate lymphoid follicle activation in the spleen and an increase in the absorption area of the ileum. High levels of RL resulted in low counts of spermatogenic cells.

KEY WORDS: rabbits / growth performanceblood profiles / histology of organs

In recent years the interest of scientists has moved from antioxidant vitamins to antioxidant phytochemicals, which can protect humans against a wide range of diseases and thus increase life expectancy [Yeung *et al.* 2018, 2019]. Also in animal production antioxidant phytochemicals are being studied due to their ability to reduce oxidative stress [Attia and Al-Harthi 2017, Islam *et al.* 2018]. However, the phytogenic composition may be highly variable depending on the botanical origin, processing method, agronomic and environment factors [Windisch *et al.* 2008]. Dorman *et al.* [2003] showed that some plants identified as phytochemicals sources possessed powerful antioxidant properties. One of these plants is milk thistle (*Silybum marianum*, family Compositae). The active compound contained in milk thistle is silymarin (a flavonolignan complex), which represents around 65-80% of the milk thistle seed extract [Kroll *et al.* 2007]. Silymarin is an excellent antioxidant, scavenging reactive oxygen species (ROS) and inhibiting lipid peroxidation, thereby protecting cells against oxygen species (OS) [Suksomboon *et al.* 2011]. Oral administration of milk thistle seed extracts in repeated doses decreased the liver enzyme activity in rats, thus confirming that milk thistle seeds are sources of a potent free radical scavenger [Ramadan *et al.* 2011, Tewari *et al*. 2017]. Milk thistle seed extract protects the liver against non-alcoholic fatty diseases [Pferschy-Wenzig *et al.* 2014, Wang *et al.* 2015].

Another important plant is *Rosmarinus officinalis* L. (common name rosemary; family Labiatae), rich in active metabolites such as caffeic acid and its derivatives, e.g. rosmarinic acid [Herrero *et al.* 2005]. Rosmarinic acid exhibits antioxidant effects and it is well-absorbed in the gastrointestinal tract and through the skin. Rosmarinic acid reduces the production of leukotriene B_4 in human polymorphonuclear leucocytes and inhibits the complement system [Ramirez *et al.* 2004]. The rosemary essential oil increases hepatocyte resistance of rats against DNA-damaging oxidative agents and exhibits free radical-scavenging activity [Harvàthová *et al.* 2010]. Rosemary has potential applications as an anti-inflammatory and anti-hepatotoxic agent [Katerinopoulos *et al.* 2005] due to its high levels of phytochemical derivatives (triterpenes, flavonoids and polyphenols). The carnosol, rosmanol and epirosmanol phenolic diterpenes of rosemary inhibit lipid peroxidation [Zeng and Wang 2001]. Rosemary enhances the levels of reduced glutathione and antioxidant enzyme activities in kidneys and testes compared to aspartame [Hozayen *et al.* 2014]. Rosmarinic acid and its extracts can inhibit the proliferation of hepatic cancer cells [Vicente *et al.* 2012] and have positive effects on vascular health [Liu *et al.* 2018].

Milk thistle seeds and rosemary leaves exhibit beneficial effects in rabbits as they improve the antioxidant status, liver markers, semen quality and fertility in bucks when used at 10 and 5 g/kg, respectively [Attia *et al.* 2017]. In addition, the inclusion of *S. marianum* in the diets decreased mortality of growing rabbits under heat stress and modified some sensory characteristics of rabbit loin meat [Cullere *et al.* 2016]. Rosemary extracts have an antidiabetogenic effect in laboratory rabbits [Bakirel *et al.* 2007].

The aim of this research was to study if the antioxidant properties of milk thistle seeds and rosemary leaves are adequate to act as natural growth promoters in growing rabbit. Thus, different levels of both phytochemical sources were added to the rabbit diets and their effects on growth performance, carcass and meat traits, blood profiles and inner organs histology were studied.

Material and methods

The scientific committee of the Animal Production Research Institute, the Agriculture Research Center, the Ministry of Agriculture and Land Reclamation, Egypt, approved the experimental procedure (protocol No. 01-05-03-37).

Dried milk thistle seeds (MTS) and rosemary leaves (RL) were purchased from a local market and ground into a fine powder using an electric dry mill. Subsequently powders were stored in well-tied black plastic bags at room temperature (≈25°C). Total phenolic compounds (equivalent to Gallic acid) and antioxidant activity (equivalent to ascorbic acid) of MTS and RL were determined according to Fogliano *et al.* [1999] and Viuda-Martos *et al.* [2010], respectively.

A total of 100, twenty-eight day old V-line rabbits (sex ratio 1:1, average weight 515.1±54.1 g) were distributed among five homogeneous groups (20 rabbits/group, 5 replicates of 4 rabbits/group). The groups were fed the same basal diet, formulated to meet rabbit nutrient requirements according to NRC (1977) and de Blas and Wiseman [2003]. The ingredients of the basal diet were 10% maize, 13% barley, 3% molasses, 39.5% clover hay, 15% wheat bran, 17.5% soybean meal, 0.8% dicalcium phosphate, 0.5% limestone, 0.3% sodium chloride, 0.3% vitamin and mineral mixture and 0.1% methionine. Chemical characteristics of the basal diet were determined according to AOAC [2007], while digestible energy was calculated according to NRC [1977]. The basal diet was not supplemented with antibiotics and *coccidiostats*.

Along the period of 28-91 days of age, the 1st group (control) received no supplementation of MTS and RL, the 2nd and the 3rd groups were supplemented with MTS at 5 and 10 g/kg, while the 4th and 5th groups were supplemented with RL at 5 and 10 g/kg, respectively. The pelleted diet (0.62 cm length and 0.45 cm diameter) and fresh water were offered ad libitum.

The rabbits were individually housed in galvanized wire cages (30×25×30 cm) and kept under similar management (environmental temperature, humidity, stocking density) and hygienic conditions (vaccinations and health care). Environmental

temperature and humidity were recorded along the trial and the temperature–humidity index (THI) was calculated according to Marai *et al.* [2001]. Rabbits were illuminated with a 16 h light/d. The rabbits were weighed at 28 and 91 d of age, while feed intake was calculated in this period from the difference between the weights of the offered feed and leavings. Feed conversion ratio was computed as the ratio between feed intake and weight gain during 28-91 days of age. Economic efficiency was calculated using an input-output analysis as reported by Attia *et al.* [2015].

A digestibility trial was performed at 8 weeks of age on 10 male rabbits per treatment (2 per replicate) individually housed in metabolic cages that allow feces collection. Quantitative collection of faces started 24 h after offering the daily feed. Feces of each rabbit were collected once a day at 9.00 am and feed intake was recorded every day in the morning for five days as the collection period. The collected samples of feces and feeds were pooled and stored at -18ºC until analysis. Fecal samples were dried at 60º C for 72 h and ground through a 1 mm screen on a Wiley grinder. Digestibility coefficients were determined and expressed on DM basis using the ((nutrient intake- nutrient voided=retained)/intake)*100. Feed and feces samples were analysed for moisture, ash, CP, EE and CF according to AOAC [2007]. NFE was calculated from the difference between dry matter and other components.

At 91 d of age, ten rabbits/group (2 per replicate) were randomly selected, fasted for 6 hours, individually weighed and slaughtered (to complete bleeding). Dressing percentage was calculated on eviscerated carcasses and organ weight (liver, kidney, spleen, heart, lungs and testes) was referred to body weight.

Samples of meat were individually taken from each rabbit leg. The Water Holding Capacity (WHC) and tenderness were measured according to Volovinskaia and Kelman [1962], pH value was measured using a pH meter [Aitken *et al.* 1962]; color intensity was determined according to Husani *et al.* [1950], while chemical composition was assayed according to AOAC [2007].

Samples of blood were collected from an air vein of 5 rabbits per group at 56 and 91 d of age and immediately stored in ice. The samples were collected from the same animals after they have been selected randomly. The blood was collected in clean tubes with or without heparin to collect plasma and serum, respectively. Blood plasma and serum were obtained by centrifugation at 860x g for 20 min at 4ºC and stored at -60ºC. Blood profiles were determined according to Attia *et al.* [2015]. Globulin content was calculated as the difference between total protein and albumin. Very low density lipoprotein was estimated by Friedwald *et al.* [1972]. Total antioxidant capacity (TAC) was measured according to Erel [2004]. The lipid peroxidation biomarker such as malondialdehyde (MDA) was assayed in the blood serum [Conti *et al*. 1990].

The liver, kidney, testes, spleen and the ileum were collected from the slaughtered animals for microscopic examination. The collected specimens were fixed in 10% neutral buffered formalin solution for at least 24 hrs. After fixation the specimens were washed in tap water and then passed through the routine paraffin embedding technique (dehydration in ascending gradients of ethyl alcohol, clearing in a series of xylene

and then passed through a series of melted paraffin wax, embedded and put in paraffin blocks). Later, the paraffin blocks were subjected to microtomy to prepare paraffin sections of 3-5 microns in thickness, which were stained with Mayer's hematoxylin and eosin according to Culling *et al*. [1975] and examined under a light microscope.

Data were analysed using the GLM procedure of statistical analysis software (SAS) version 6.11 [SAS Institute 1996]. The mean difference at $p<0.05$ was tested using the Student-Newman-Keuls test. In addition, orthogonal contrast analysis was performed to test differences between the control group and the MTS groups, the control and the RM groups as well as the MTS and RL groups. Chi square statistics was used to evaluate the effect of experimental treatments on mortality rate. When statistical analysis show no differences, data were not reported in the tables.

Results and discussion

The determined chemical characteristics of the basal diet, on as a fed basis, were: 90.3% dry matter, 80.8% organic matter, 17.2% crude protein, 13.5% crude fibre, 2.8% ether extract, 9.5% ash and 57.0% NFE. The calculated digestible energy value was 2,464 kcal/kg diet.

The average temperature and relative humidity during the experimental period were 25.8°C and 67.7%, respectively, with a temperature-humidity index (THI) of 28.1.

MTS had greater total polyphenol conents equivalent to gallic acid as mg/100g dry matter (392.1±5.6 vs. 174.7±9.5) and higher antioxidant activity equivalent to ascorbic acid mg/g dry matter (780±84.9 vs. 565±21.2) than rosemary leaves.

The effect of different dietary treatments on growth performance and nutrient digestibility of rabbits during 28-91 d of age is presented in Table 1. The MTS and RL groups showed greater ($p<0.01$) growth than the control, while the MTS 10 g/kg group showed a greater body weight gain (BWG) than the group fed the same dose of RL. Groups supplemented with 5g/kg of each herb significantly improved growth parameters compared to the control.

Group supplemented with MTS showed a higher ($p<0.05$) feed intake than the control and 5g/kg RL groups. Feed conversion ratio (FCR) significantly improved due to MTS and RL supplementations compared to the control group. MTS was more effective ($p<0.01$) than RL in improving FCR at 10g/kg, while the opposite happened at 5 g/kg of diets. Groups on MTS consumed more water than the control ($p<0.01$) and RL ($p<0.05$) groups. Economic efficiency was the greatest in the RL 5 g/kg group. The CP digestibility was higher in the experimental groups than in the control, except for RL 10g/kg. MTS 10 g/kg and RL 5 g/kg similarly improved ($p<0.01$) CP digestibility than MTS 5g/kg group. OM digestibility of the MTS 10 g/kg and RL 5 g/kg groups was higher ($p<0.01$) than in the control and RL 10 g/kg groups, while DM digestibility was similarly increased ($p<0.01$) in to different dietary treatments when compared to the control.

Table 1. Effect of milk thistle seeds and rosemary leaves on growth performance, economic efficiency and nutrient digestibility of growing V-line rabbits during 28- 91 d of age

Herb type	Dose (g/kg)	Initial body weight (g)	Body weight gain (g)	Feed intake (g)	Feed conversion	Water intake (cm)	Economic efficiency (%)	CPd	OMd	DMd	NFEd
Control		520	1448^{c}	98^{b}	4.68^{a}	168^{b}	34.3^{b}	75.77^{c}	71.22^{b}	67.71^{b}	78.38
MTS	5	521	1614^{ab}	111^{a}	4.44^{b}	220^{a}	31.3^{b}	77.33^{b}	71.87^{ab}	69.18^{a}	78.58
	10	504	1675^{a}	113^{a}	4.26^{c}	219^{a}	34.0^{b}	78.57^{a}	72.32^{a}	69.80^{a}	78.85
RL	5	515	1588^{ab}	96^{b}	4.28^{c}	178^{b}	49.3^{a}	78.56^{a}	72.48^{a}	69.76^{a}	79.05
	10	513	1554^{b}	105^{ab}	4.53^{b}	174^{b}	33.5^{b}	76.21^{c}	71.61^{b}	69.89^{a}	78.88
P-value		0.878	0.0001	0.039	0.0001	0.0001	0.0001	<.0.001	0.0002	<.0.001	0.255
RMSE		55.04	131	20.48	0.139	37	3.96	1.01	0.62	0.92	0.72
Control vs.MTS		0.615	<.0001	0.014	<.0001	<.0001	0.001	<.0.001	0.001	<.0.001	0.232
Control vs. RL		0.692	0.002	0.590	<.0001	0.406	0.001	0.0002	0.001	<.0.001	0.040
MTS vs. RL		0.617	0.642	0.245	<.0001	0.035	0.001	<.0.003	0.033	0.101	0.188

^{abc}Means in a column bearing different superscripts are significantly different. RMSE – root mean square error. MTS – milk thistle seeds; RL – rosemary leaves; CPd – crude protein digestibility; NFEd – nitrogen free extract digestibility; OMd – organic matter digestibility; DMd – dry matter digestibility.

Table 2. Effect of milk thistle seeds and rosemary leaves on blood cell traits of V-line growing rabbits during 28-91 d of age

Herb type	Dose (g/kg)	RBC ($\times10^{6}$/mL)	PCV (%)	Hgb (g/dL)	MCHC (g/dL)	WBC ($\times10^{3}$/mL)	Lym (%)
Control		6.33^{b}	41.40	10.30	24.86	6.40	42.6^{b}
MTS	5	6.52^{a}	42.40	10.80	25.46	6.30	44.6^{a}
	10	6.49^{a}	42.00	10.90	25.93	6.28	44.5^{a}
RL	5	6.56^{a}	43.00	11.10	25.83	6.33	44.0^{a}
	10	6.60^{a}	43.20	11.30	26.14	6.33	42.5^{b}
P-value		0.004	0.214	0.127	0.257	0.760	0.013
RMSE		0.156	1.89	0.864	1.35	0.211	1.71
Control vs.MTS		0.006	0.280	0.107	0.118	0.184	0.005
Control vs. RL		0.0001	0.025	0.010	0.037	0.396	0.332
MTS vs. RL		0.121	0.162	0.306	0.730	0.951	0.601

^{abcd}Means in a column bearing different superscripts are significantly different. RMSE – root mean square error; MTS – milk thistle seeds; RL – rosemary leaves; RBC – red blood cell counts; PCV – packed cell volume; Hgb – haemoglobin; MCV – mean cell volume; MCH – mean cell hemoglobin concentration; MCHC – mean cell hemoglobin concentration; WBC – white blood cell counts; Lym –lymphocytes.

Table 3. Effect of milk thistle seeds and rosemary leaves on liver and renal functions of growing V-line rabbits during 28- 91 d of age

Herb type	Dose (g/kg)	Liver function			Kidney function			TP (g/dl)	Alb (g/dL)	Glob (g/dL)	Alb/ Glob ratio
		ALT (IU)	AST (IU)	AST/ ALT	urea (mg/dl)	creatinine (mg/dl)	Ur/Cr				
Control		36.02^{a}	53.50^{a}	0.673^{a}	46.10	1.09	42.32	5.08	2.64	2.44	1.13
MTS	5	31.08^{b}	52.62^{ab}	0.591^{b}	45.75	1.05	43.50	5.36	2.73	2.63	1.07
	10	29.44^{b}	52.52^{ab}	0.560^{b}	45.68	1.06	43.28	5.74	2.93	2.81	1.05
RL	5	29.44^{b}	50.48^{bc}	0.58^{b}	43.05	1.04	41.46	5.38	2.61	2.77	0.98
	10	28.86^{b}	50.06^{c}	0.577^{b}	43.90	1.03	42.44	5.76	2.92	2.84	1.05
P-value		<0.0001	0.003	0.0002	0.084	0.143	0.645	0.090	0.601	0.516	0.945
RMSE		1.60	1.37	0.032	1.94	0.036	2.30	0.418	0.413	0.400	0.274
Control vs.MTS		<.0001	0.228	<.0001	0.721	0.091	0.406	0.053	0.411	0.216	0.660
Control vs. RL		<.0001	0.0003	<.0001	0.022	0.012	0.772	0.045	0.590	0.111	0.465
MTS vs. RL		0.076	0.150	0.280	0.206	0.833	0.381	0.393	0.848	0.485	0.728

^{abc}Means in a column bearing different superscripts are significantly different. RMSE – root mean square error. MTS – milk thistle seeds; RL – rosemary leaves; ALT – alanine amino transferase; AST – aspartate amino transferase; Ur/Cr – urea/creatinine; TP – total protein; Alb – albumen; Glob – globulin.

Table 4. Effect of milk thistle seeds and rosemary leaves on antioxidant indices and blood biochemical constituents of growing V-line rabbits during 28- 91 d of age

Herb type	Dose (g/kg)	TAC (mm/L)	MDA (mm/L)	Glu (g/dl)	TL (mg/dl)	Chol (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dL)	Triglycerides (mg/dl)	T3 (ng/ml)	T4 (ng/ml)	T3/T4 ratio
Control		1.40	2.95	87.82	312.4	115.5^{a}	64.38^{a}	23.41	27.72^{bc}	117.0	1.23^{ab}	2.23^{b}	0.552^{a}
MTS	5	1.87	2.76	82.16	315.8	119.5^{a}	65.86^{a}	23.34	30.26^{ab}	116.7	1.18^{b}	2.31^{ab}	0.510^{c}
	10	1.93	2.79	80.90	315. 8	118.8^{a}	61.46^{a}	23.26	34.06^{a}	116.3	1.21^{ab}	2.33^{a}	0.519^{bc}
RL	5	1.73	2.83	82.24	314.3	114.3^{a}	65.58^{a}	22.73	25.94^{bc}	113.6	1.24^{a}	2.30^{ab}	0.541^{ab}
	10	1.83	2.88	85.48	296.9	100.4^{b}	54.12^{b}	23.08	23.16^{c}	115.4	1.27^{a}	2.32^{ab}	0.548^{a}
P-value		0.143	0.595	0.480	0.037	0.0003	0.004	0.970	0.002	0.970	0.006	0.058	0.001
RMSE		0.340	0.202	6.70	10.1	5.92	4.71	1.69	3.72	8.45	0.054	0.080	0.024
Control vs.MTS		0.014	0.127	0.102	0.545	0.274	0.783	0.908	0.041	0.908	0.083	0.005	0.003
Control vs. RL		0.055	0.395	0.294	0.235	0.021	0.094	0.593	0.135	0.594	0.220	0.014	0.428
MTS vs. RL		0.815	0.649	0.874	0.888	0.372	0.375	0.712	0.900	0.712	0.020	0.872	0.039

^{abc}Means in a column bearing different superscripts are significantly different. RMSE – root mean square error; MTS – milk thistle seeds; RL – rosemary leaves; TAC – total antioxidant capacity; MDA –malondialdehyde; LDL – low density lipoprotein; VLDL – very low density lipoprotein; HDL – high density lipoprotein.

Table 5. Effect of milk thistle seeds and rosemary leaves on carcass and meat characteristics of growing V-line rabbits during 28-91 d of age

Herb type	Dose (g/kg)	Dressing	Liver	Heart	Lung	Dry matter (%)	Crude protein (%)	Ether extract (%)	Ash (%)	pH
Control		52.8^{b}	3.28^{a}	0.270^{ab}	0.728^{a}	28.10	22.52	3.796	1.24	5.33^{c}
MTS	5	55.0^{ab}	3.30^{a}	0.306^{a}	0.566^{b}	28.40	22.80	3.800	1.31	5.40^{bc}
	10	56.8^{a}	2.88^{b}	0.258^{b}	0.634^{ab}	29.06	23.12	3.876	1.42	5.45^{abc}
RL	5	54.0^{ab}	3.08^{ab}	0.284^{ab}	0.604^{ab}	29.12	23.34	3.91	1.39	5.49^{ab}
	10	52.2^{b}	3.14^{ab}	0.277^{ab}	0.611^{ab}	29.14	23.30	3.94	1.29	5.57^{a}
P-value		0.017	0.011	0.027	0.037	0.501	0.530	0.605	0.067	0.002
RMSE		2.06	0.18	0.022	0.077	1.16	0.866	0.172	0.100	0.080
Control vs.MTS		0.013	0.074	0.321	0.006	0.332	0.365	0.661	0.037	0.043
Control vs. RL		0.787	0.107	0.379	0.009	0.120	0.107	0.190	0.081	0.0002
MTS vs. RL		0.740	0.005	0.008	0.220	0.3145	0.4056	0.336	0.010	0.110

^{abc}Means in a column bearing different superscripts are significantly different. , RMSE – root mean square error. MTS – milk thistle seeds; RL – rosemary leaves.

Table 6. Results of microscopic examination for morphological characteristics of liver, kidney, spleen, testes and ileum

Group/organs	Liver	Kidney	Spleen	Testis	Ileum
10 g/kg MTS	N	N	B – moderate lymphoid follicles activations (Fig. 1)	N	B – increased the absorption
5 g/kg MTS	N	N	B – activation of melanomacrophage center (Fig. 2)	B – high spermatogenesis (Fig. 6)	B – increase the absorption area
10g/kg RL	N	N	B – hyperactivation of lymphoid tissues of white pulp (Fig. 4)	D – low spermatogenesis (Fig. 5)	N
5 g/kg RL	N	N	B – enlarged melanomacrophage center by a greater enlargement of red pulp with melanomacrophage centers (Fig. 3)	N	B – increase the absorption area (Fig. 7)

N – the treatment has no effect on the examined organs. B – the treatment has a favourable effect on the examined organs. D – the treatment has a harmful.

The effect of different dietary treatments on blood cell traits of rabbits is presented in Table 2. There was an increase ($p<0.01$) in RBCs due to MTS or RL supplementations compared to the control group. Contrast analysis showed that the RL groups had higher values of PCV, MCHC ($p<0.05$) and Hgb ($p=0.01$) than the control. Lymphocyte count was significantly increased with MTS and RL at 5g/kg compared to the other groups.

The effect of different treatments on liver and renal functions as well as plasma protein of rabbits is presented in Table 3. There were significant decreases in ALT (alanine aminotransferase) and ALT/AST (aspartate aminotransferase) ratios due to supplementations with MTS and RL ($p<0.01$) compared to the control group. Groups fed RL diets had a lower ($p<0.01$) AST value than the control. RL at 5 g/kg decreased plasma urea and creatinine ($p<0.05$) compared to the control group.

The effect of different dietary treatments on antioxidants indices and blood biochemical constituents of rabbits is presented in Table 4. TAC increased in the MTS ($p< 0.05$) groups compared to the control. In turn, RL decreased ($p<0.05$) plasma cholesterol levels compared to the other groups. A similar trend was observed in LDL and this coincided with the numerical decrease in plasma total lipids. MTS at 10 g/kg increased HDL (high-density lipoprotein) compared to the control group and the RL groups ($p<0.01$). The MTS and RL groups showed higher T4 than the control ($p<0.01$; $p<0.05$; respectively). The RL groups exhibited higher T3 than the MTS groups ($p<0.05$). In particular, groups supplemented with RL showed higher T3 values compared to the 5 g/kg MTS group. MTS increased T_3/T_4 compared to the control ($p<0.01$) and RL groups ($p<0.05$). The group supplemented with 5 g MTS showed a higher T_3/T_4 ratio compared to the other groups except for the MTS 10g/kg group. The latter group had a significantly higher T_3/T_4 ratio compared to the other groups except for RL 5 g/kg.

The effect of different dietary treatments on carcass and meat traits is presented in Table 5. MTS increased ($p<0.05$) dressing percentage compared to the control group and MTS at 10 g/kg diet increased dressing percentage compared with the control and RL at 10 g/kg. Groups fed RL diets had a higher ($p<0.01$) liver percentage compared to MTS. Liver percentage decreased in the group supplemented with a high dose of MTS compared to the low dose. Heart percentage was higher ($p<0.01$) in the MTS than RL groups, 5 g of MTS increased heart % compared with the high dose of the same herb. Lung percentage increased in the control group compared to the MTS and RL groups. Contrast analysis showed that the MTS groups had a higher ash content in meat than the control. The pH value increased in the MTS ($p<0.05$) and RL ($p<0.01$) groups compared to the control, especially in the RL 10 g/kg group diet compared to the control and MTS 10 g/kg. The group fed 5 g RL had a higher meat pH than the control group.

The results of organ morphology examinations are shown in figures 1-7 and the major findings are summarized in Table 6.

Administration of MTS or RL at doses of 5 and 10 g/kg throughout the experimental period resulted in a normal morphology of the liver and kidneys (figures not shown);

however, the high dose of MTS resulted in moderate lymphoid follicle activations in the spleen (Fig. 1). Meanwhile, administration of the MTS low dose induced an activation of the melanomacrophage center in the spleen (Fig. 2). The elongation of intestinal villi leads to an increase in the absorption area (Fig. 7).

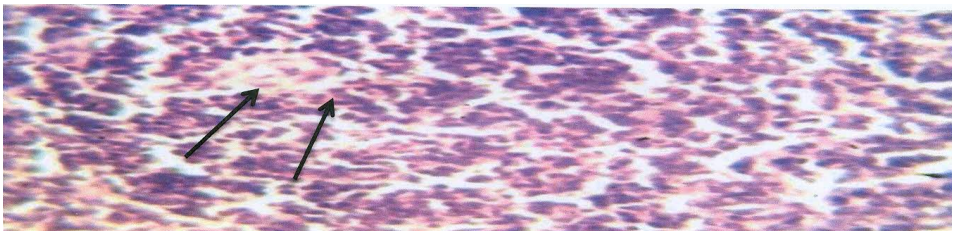

Fig. 1. Spleen section from the MTS 10 % group showing moderate lymphoid follicles activations (Arrows) (H & E ×200).

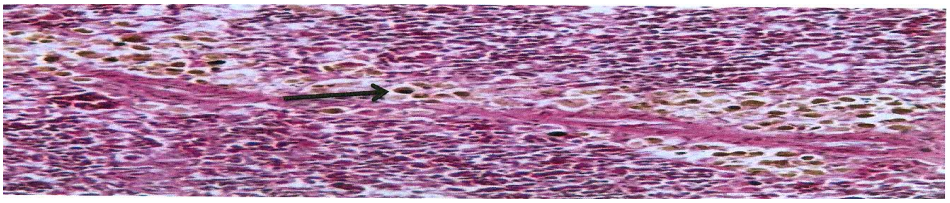

Fig. 2. Spleen section from MTS 5 % group showing activation of melanomacrophage center (Arrow) (H&E X 40).

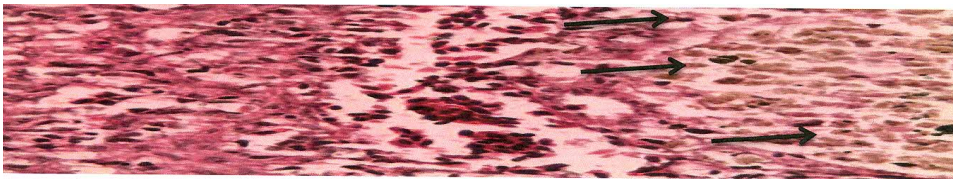

Fig. 3. Spleen section from RL 5 % group showing enlarged melanomacrophage center by higher magnification of red pulp with Melanomacrophage centers (MNCs) infiltration (Arrows) (H&E X400).

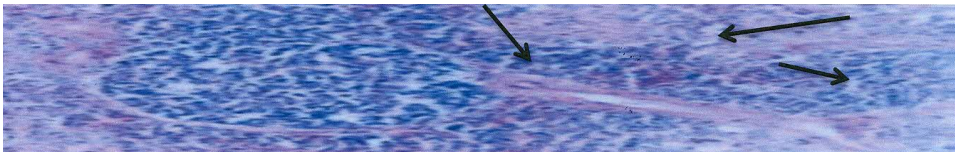

Fig. 4. Spleen section from RL 10 % group showing hyper activation of lymphoid tissues of white pulp (Arrows) (H & E, X 200).

The high dose of RL resulted in low counts of spermatogenic cells (Fig. 5 and 6). In addition, the spleen showed hyperactivation of lymphoid tissues in the white pulp (Fig. 4). The low dose of RL induced spleen enlarged melano-macrophage center by a greater enlargement of the red pulp with melanomacrophage centers (Fig. 3).

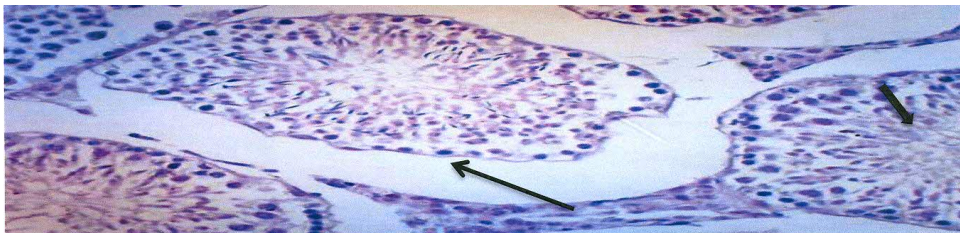

Fig. 5. Testis section from RL 10 % group showing increased the space between seminiferous tubules and activation of Leydig cells, which leads to lower space of seminiferous tubules and low spermatoginic cells (Arrows). HXE X200.

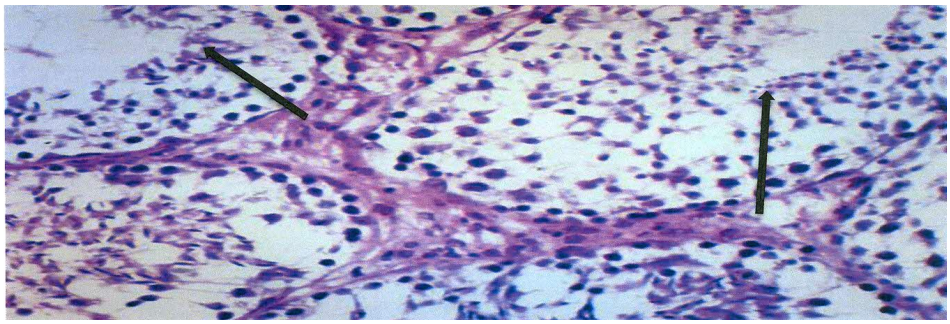

Fig. 6. Testis section from MTS 5 % group showing highly increases in spermatogenic cells and appearance of Sertoli cells (Arrow) and Primary Spermatogonial cells (Arrow) (H&E 400).

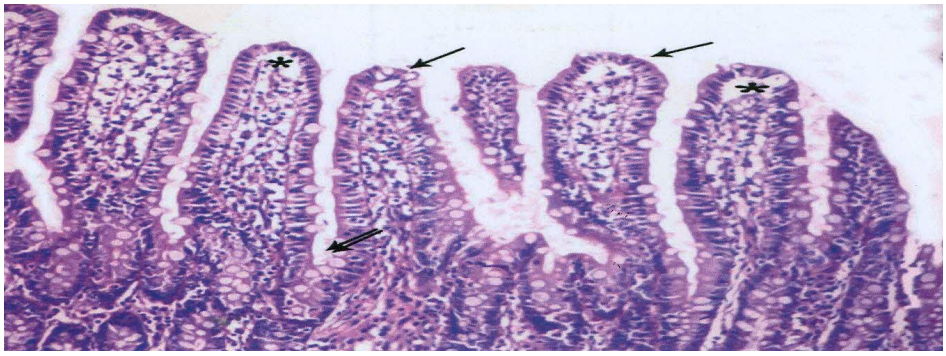

Fig. 7. Ileum tissue section from RL 5 % group showing finger like projections of the villi and numerous numbers of goblet cells (↑), crypts of Leiberkuhn between the bases of the villi (↑↑) and small villi subepithelial spaces (*). H&E. X 200.

The present results demonstrated that MTS at 10 g/kg diet improved growth rate, FCR, feed and water intake in growing rabbits, resulting in an increased dressing percentage of carcasses. These effects may be attributed to improved CP, OM and DM digestibilities as well as an improved function of the immune system suggested by the

increased proportion of lymphocytes. The increase in HDL and plasma T4 associated to a decreased AST/ALT ratio and liver % also indicated an improved liver function in the MTS 10 g/kg group. The histological examination of the ileum showed an increased absorptive capacity of animals fed the highest dose of MTS. In agreement with the present results, Tedesco *et al.* [2004] reported that the addition of *S. phytosome* (a complex of silymarin and phospholipids) at 600 mg/kg increased broilers' body weight by 14.83% in relation to the control. This increase was lower than that (31.12%) reported by Chand *et al.* [2011], who used *S. marianum*. A significant improvement of BWG due to MTS was attributed to the antioxidant activity that stimulated protein synthesis by the bird's enzymatic system [Makki *et al.* 2013]. The exact mechanism for improving body weight is not well established; however, this effect might be due to the saving of energy from maintenance resulting from an improved immune function of the birds receiving MTS in the current study. Similarly as in the present findings, Chand *et al.* [2011] reported that MTS improved feed intake, FCR and dressing percentage in broilers fed an aflatoxin B1 contaminated-diet. In turn, Tedesco *et al.* [2004] reported +22.3% of feed intake in birds fed an aflatoxin B1 contaminated diet and supplemented with MTS compared to the control. Hasheminejad *et al.* [2015] demonstrated that MTS reduced the toxic effects of AFB_1 and the metabolic demands of the intestinal tract in broiler chickens. Furthermore, 0.5% *S. marianum* in diets reduces pathogenic bacteria in the ileum [Kalantar *et al.* 2014]. The major mechanism of action in the case of medicinal plants is connected with the adhesion of bacterial membranes, which inhibits bacterial enzymes activation [Stiles *et al.* 1995].

The increased cellular immunity found herein are in agreement with the results reported by Chand *et al.* [2011] and Makki *et al.* [2013], who found that the relative weight of the lymphoid organs (bursa, spleen and thymus) and antibody titers against ND, IB and IBD were improved in the MTS fed group. This demonstrated an increased immune function due to MTS supplementation, as seen in the increased spleen lymphoid follicle activations.

The present investigation showed that RL at 5 g/kg improved BWG, FCR, digestibility coefficients of CP, OM and DM as well as economic efficiency compared with RL at 10 g/kg. Also, Singletary and Rokusek [1999] found improvements in the growth performance of broilers fed diets supplemented with rosemary and this concurred with an improvement in digestibility of most nutrients in comparison to the control. Rostami *et al.* [2015] showed that broilers fed 1.0% rosemary powder (RP) exhibited lower ($p< 0.05$) weight gains and final weights than those fed 0.5%. Norouzi *et al.* [2016] showed that FCR of broilers was similarly improved in the groups supplemented with rosemary at 0.5, 1, 1.5% of the diet compared to the control, suggesting that 0.5% is a sufficient level.

These improvements could be attributed to the antimicrobial and antioxidant properties of rosemary [Lopez-Bote *et al.* 1998]. Rosemary at 1% may be used as an antimicrobial agent in the intestinal tract for broiler chicks [Ghalib *et al.* 2008]. The positive effect of rosemary on decreasing *E. coli* in the intestinal tract could improve the

animal health and performance, as described by Tollba [2010]. This author described the mechanism of the bacterial inhibition effect of aromatic plants as an interference between the contents of rosemary and cellular membranes of microorganisms, which led to a change in the diffusion of potassium ions and hydrogen, affecting the viability of microorganisms. In 42 d old broilers supplemented with 500 mg of rosemary, Manafi *et al.* [2014] found an improved feed intake and FCR compared to the control. This was attributed to the positive effects of rosemary on nutrient digestibility, as reported by Alcicek *et al.* [2003] and Hernandez *et al.* [2004]. Essential oils and their mixture could positively affect the intestinal microflora [Lee *et al.* 2003]. In the human, polyphenols may have a modulatory role in cardiovascular diseases and cancer [Yeung *et al.*, 2019].

Similarly as in the present findings, Tollba [2010] found that relative weights of carcass, giblets and dressing percentage of broilers fed rosemary were improved compared to the control. In turn, Al-Shuwaili [2014] showed no differences in carcass percentages of chicks fed different concentrations of rosemary, thyme or their mixture compared to the control. In a study on quails, a rosemary plus oregano volatile oil mixture enhanced the levels of lymphocytes and neutrophils [Yesilbag *et al.* 2012], similarly to the current study. On the other hand, Savoini *et al.* [2003] reported that a dietary rosemary extract markedly decreased the counts of WBC and blood neutrophil percentage compared to the control in organically managed dairy goats. This contradiction could be attributed to the effect of animal species and age, as well as to the rosemary level in the diet.

The present results indicated that different levels of MTS and RL are safe and might boost liver and renal functions. This result is in agreement with the results reported by Muhammad *et al.* [2012], who found lower levels of serum enzymes such as alkaline phosphatase (ALP), AST and ALT in the groups fed diets containing aflatoxin or isoniazid (a substance inducing hepatotoxicity) and supplemented with MTS. The decrease in serum urea due to RL indicated an improvement in renal function. This finding is in agreement with the study by Ayaz [2012], who reported that treatment of diabetic animals with 200 mg/kg/d rosemary inhibited the increase of BUN, serum creatinine and uric acid in comparison to untreated diabetic animals. The excellent recovery of renal function after supplementation with streptozotocin (a diabetic inducer) in rats expected with treatment of rosemary may be explained by the regenerative capability of renal tubules. The reduction of urea and creatinine levels is line with the finding of Abid Ali *et al.* [2015] that silymarin extract and legalon supplementation resulted in a remarkable protective effect against nickel chloride, which reversed the levels of urea and uric acid near the normal.

It is interesting that MTS and RL significantly increased TAC, while numerically decreased MDA compared to the control. We also find that RL decreased total lipid, total cholesterol and LDL cholesterol levels when added at 10g/kg diet, while MTS at the same level increased HDL. These results suggested the potential of MTS and RL as antioxidants and as cholesterol-lowering agents. In agreement with the

present study, Tollba [2010] found that total lipids and cholesterol were significantly ($p<0.05$) decreased due to the effect of rosemary supplementation. Similarly, total cholesterol and lipid levels were significantly decreased due to the administration of a diet containing 1% rosemary to broilers [Ali *et al.* 2008]. Also, Alagawany and Abd El-Hack [2015] showed that the diet enriched in rosemary at 3, 6 and 9 g/kg reduced serum triglycerides and total cholesterol, as well as LDL-cholesterol concentrations, whereas HDL-cholesterol concentrations were elevated with the same addition. Bölükbaşi *et al.* [2008] reported that rosemary dietary supplementation of laying hen significantly depressed serum triglyceride and total cholesterol levels. In the same context, Rahimi *et al.* [2011] pointed out that blood triglyceride, total and LDL-cholesterol concentrations were significantly reduced by an addition of phytogenic feed additives to chicken diets, while HDL-cholesterol concentrations increased. In contrast, Abd El-Latif *et al.* [2013] found that an addition of rosemary to chicken diets increased serum triglycerides, total cholesterol and LDL-cholesterol. In turn, Osman *et al.* [2010] noted that rosemary added at 0.5 and 1 g/kg diet had no effect on serum concentrations of protein, albumin, creatinine or cholesterol. Medicinal plants or their products affected blood lipid parameters in different ways [Alagawany *et al.* 2015]. Hyperlipidaemic effects were reported in the case of certain herbal plants [Alagawany *et al.* 2015], whereas hyperlipidaemia was observed when others were administered [Farag *et al.* 2014]. The discrepancies between these studies might be due to the differences in the phytogenic feed additives used, product type (powder, essential oil, etc.), doses and type of administration, as well as experimental conditions.

The hypocholesteromic effect of MTS was demonstrated by Kreeman *et al.* [1998], who concluded that silymarin in milk thistle seeds given to rats with diet-induced hypercholesterolemia demonstrated an anticholesterolemic effect manifested as an increase in HDL cholesterol and a decrease in total and biliary cholesterol levels. Suksomboon *et al.* [2011] showed that MTS, with its antioxidant action, might prove beneficial for people at risk of high cholesterol levels and diabetes. Similarly, Ramadan *et al.* [2011] reported that flavonoids of milk thistle seeds had potent antioxidant effects, as indicated by significant increases of superoxide anions and lipid oxygen radicals due to lipid peroxidation [Shaker *et al.* 2010]. The latter authors demonstrated that the antioxidant activity of MTS *in vivo* is related with an increased content of glutathione, which detoxifies an array of hormones, drugs and chemicals. Müzes *et al.* [1991] reported that silymarin increased the level of superoxide dismutase in cell cultures. Separate and combined treatments with silymarin and vitamin C significantly reduced ($p<0.05$) the level of TBARS and increased ($p<0.05$) the activities of SOD, CAT, GPx and GST in livers of hepatotoxic rats when compared to the normal [Sabiu *et al.* 2015].

There was a significant effect of MTS and RL only on RBCs compared to the control without any effect of herb type or level. PCV, Hgb, MCV, MCH and MCHC were not significantly affected by dietary treatments. The results indicate that MTS and RL are safe feed additives for growing rabbits. Similarly, Ahmad *et al.* [2012] observed that birds kept on silymarin, vitamin E and their combinations showed

hematobiochemical responses similar to those of the control group. On the other hand, chickens fed silymarin, vitamin E or their combination plus 1000 μg/ochratoxin showed improvement in hematobiochemical responses (leukocyte count, Hgb and PCV). Nevertheless, no significant differences were observed for Hgb, PCV and RBC between treatments with varying levels of the rosemary extract compared to the control [Tollba 2010]. Yesilbag *et al.* [2012] in their experiments on quails given 100 mg of rosemary per kg of diet showed an increase in the levels of RBCs, Hgb and PCV.

Zahid and Durrani [2007] reported a 3.92% improvement in the dressing percentage, higher breast and thigh weights in broilers fed 15g/kg MTS. These improvements were greater than those reported by Chand *et al.* [2011] with the 10g/ diet supplementation. On the other hand, Makki *et al.* [2013] demonstrated that the percentages of thigh, back, neck, wings and legs in broilers were not influenced by different levels of AFB1 and Milk thistle seeds. Tollba [2010] found significant improvements ($p<0.05$) in the relative weight of the carcass, giblets and dressing percentage of broilers fed experimental additives (citric acid, lactose and rosemary) compared to the control.

Conclusions

Milk thistle seeds and rosemary leaves have a beneficial effect on growth performance of young rabbits. In particular, 10 g/kg of MTS and 5 g/kg of RL improved growth rate, nutrient digestibility (crude protein, organic matter and dry matter), enhanced immunity, TAC, total lipid, total cholesterol, as well as liver and kidney function. In turn, RL seemed to be the best supplement due to its low dose necessary to reach similar results, thus giving a greater economic benefit.

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