



EFFECTS OF MORINGA LEAF AS A NATURAL ANTIOXIDANT ON GROWTH PERFORMANCE, BLOOD LIPID PROFILES AND IMMUNE RESPONSE OF RABBITS UNDER MODERATE HEAT STRESS

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ABSTRACT: Heat stress has negative effects on the performances of growing rabbits. The rabbit sector is a more vulnerable to global warming and climate change. The objective of this paper was studying effects of moringa leaf meal (MLM) on performance, immune response, and blood lipid profiles of growing rabbits under moderate heat stress. Forty-eight V-line unsexed rabbits, 5 weeks old, with initial weight of 704.4 ± 68.19 g were used and allocated randomly to four groups of 12 rabbits each. First group fed basal diet free of feed additives (control). Second, third and fourth groups fed basal diet with 0.1, 0.2 and 0.3 % MLM supplementation, respectively. The results revealed that final body weight was significantly increased by the inclusion of MLM at 0.2 and 0.3 % compared with control. There was significant decrease in daily feed intake with an increase in the rate of MLM up to 0.2 %. Moringa significantly increased antibody titers against SRBCs compared with control group at 7, 14 and 21 days after vaccination. Also, it stimulated significantly IgM immune response of growing rabbits in comparison with the control group. Serum cholesterol, LDL and MDA levels were significantly decreased as MLM concentration administered increases. Significant increase in total antioxidant capacity was recorded due to MLM supplementation in comparison with control. Based on the experimental results, it is concluded that supplementing rabbits under moderate heat stress with MLM could be better strategy to improve immune response and blood lipid profiles.

Key words: Rabbits – Moringa - Antioxidant status - Hematological parameter.

INTRODUCTION

Global warming has a great impact on the productive status of rabbits. It has risen the earth surface temperature by about 0.7°C since the early 20th century. It is expected that the global temperature rise will reach 1.8-4°C by 2100 (IPCC, 2014). Also, Heat stress can cost rabbit farmers a significant amount of money, due to production losses in meat rabbit production (Villalobos et al., 2008).

Heat stress raises lipid oxidation as a result of increasing free radical generation which increases the formation of reactive oxygen species and induces oxidative stress (Altan et al., 2003). On the other hand, Moringa Oleifera is one of the plants that contain natural antioxidants such as zeatin, kaempferol, caffeoylquinic acid, quercetin and β - sitosterol, which act as antioxidant, antimalarial, cardiac stimulant and has antibacterial and antifungal activities (Farooq et al., 2007). Additionally, the antioxidant properties of moringa leaves can be due to the existence of glycosides, tannins, anthocyanin, polyphenols and thiocarbamates, which activate antioxidant enzymes, inhibit oxidases and eliminate free radicals, (Luqmans et al., 2012). Therefore, the aim of this study was to examine the effect of various supplementation of MLM as a natural source of antioxidant on growth performance, immune response and blood lipid profiles of growing V-line rabbits under moderate heat stress.

MATERIALS AND METHODS

Animal and their management

Forty-eight growing V-line unsexed rabbits, 5 weeks old, with initial weight of 704.4±68.19 g were randomly divided into four treatments (n=12). Each treatment was sub-divided into 4 replicates (n=3). Rabbits were kept in wire batteries of 45x36x36 cm under hygienic conditions and were fed experimental diets *ad libitum* until 12 weeks of age. Four pelleted diets were prepared. The first group was fed basal diet free of feed additives (control). Second, third and fourth groups were fed basal diet with 0.1,

0.2 and 0.3 % MLM supplementation, respectively. Fresh water was automatically available at all times for each cage. The ingredients of the experimental diets are shown in Table (1) covered the nutrition requirements according to NRC (1977).

Moringa preparation

The moringa leaves were collected from a private farm of sandy soil in Borg El-Arab city, Alexandria, Egypt. The harvested leaves were air dried in shade under a shed until they were crispy to touch while retaining their greenish coloration. The dried leaves were then milled using a hammer mill (1 mm mesh) to produce MLM suitable for incorporation into rabbit's diets.

Environmental data and heat tolerance measurements

Air temperature and humidity were recorded daily by room thermometer and hygrometer. Temperature-humidity index (THI) = $db^{\circ}C - [(0.31 - 0.31 RH \%) (db^{\circ}C - 14.4)]$, whereas $db^{\circ}C$ = dry bulb temperature in Celsius and RH = relative humidity % (Marai et al., 2001). The THI values were classified as: < 27.8 = absence of heat stress; 27.8 to < 28.9 = moderate heat-stress, 28.9 to < 30.0 = severe heat stress and > 30.0 over severe heat stress. Respiration rate per minute (RpM) was measured by counting the flank movement per minute and rectal temperature (°C) was measured by clinical thermometer at mid-day (between 12 am to 3 pm) on 3rd and 6th week of the experiment.

Data collect

Rabbits have never been treated with any kind of systematic vaccination or medication. Live body weight and feed intake were recorded weekly. Also, daily weight gain and feed conversion were calculated. At the end of the feeding trial, 6 ml of blood sample was taken from 6 rabbit's ear veins of each treatment with a sterile syringe. 2 ml of the blood was put into a bijou bottle containing ethylene diamine tetraacetic acid (EDTA) for hematological assay. The remaining 4 ml of the blood sample was put into a sterile vacutainer tube without an anticoagulant for

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serum biochemical analysis. The hematological assay was carried out to determine haemoglobin (Hb) values, red blood cell (RBC) counts according to Natt and Herrick (1952), packed cell volume (PCV) and white blood cell (WBC) according to Hepler (1966). The differential count of specific types of leukocytes was according to the Pappenheim method. Serum total lipids, triglycerides, cholesterol, LDL and high-density lipoprotein (HDL), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), total antioxidant capacity (TAC) and MAD concentrations were estimated using commercial kits (Bio Merieux, France). Serum immunoglobulin IgG and IgM were determined using ELISA technique. Four rabbits of each group were immunized with 0.1 ml of a 2.5% Sheep Red Blood Cells (SRBCs) at 15 days after starting the experimental diets, to measure Antibody titer against SRBCs. Antiserum to SRBCs was collected 7, 14 and 21 days post-challenge according to Wegmann and Smithies (1966). The agglutination titer was expressed as the \log^2 (Nelson et al., 1995).

Statistical analysis

Results were expressed in the mean \pm SD. All data were analyzed using one-way analysis of variance (ANOVA) using SPSS 11.0 statistical software (SPSS, Inc., Chicago, IL, 2001). Significant differences between means were detected using new Duncan multiple range tests (Duncan, 1955).

RESULTS AND DISCUSSIONS

Meteorological parameters and heat tolerance measurements

Considering the overall mean of THI for the experimental period it is clear that rabbits were exposed to moderate heat-stress, whereas ambient temperature ranged between (28.0-31.2 °C); THI 28.1 and relative humidity was (63.3-74.8 %). Generally, body temperature is affected by many factors such as feed, environmental temperature, disease, sex and age (Guyton and Hall, 2000). The results revealed non-significant effect on respiration rate and rectal temperature (at 3rd and 6th week) due

to different treatments as compared with control (Fig. 1A&B).

Growth performance

At 12 weeks of age, (Table 3) the final live body weight has increased significantly by the inclusion of MLM at 0.2; 0.3% in comparison with control group. There was significant decrease in daily feed intake with an increase of MLM up to 0.2%. The observed results showing that inclusion of MLM improve the feed conversion ratio in comparison with the control group, but this improvement didn't reach significant level.

The positive effect of MLM on growth performance of rabbits was noticed in some previous studies, Nuhu (2010), regarded the better growth rate to protein quality and amino acids content of moringa leaves. El-Badawi et al. (2014) suggested that moringa dry leaves at level (0.15 or 0.30%) could use as a natural growth promoter. In the same time, certain adverse effect could be due to the high content of some phytochemical compounds (phenols, coumarins, alkaloids and tannins), or might be due to the fact that *M. oleifera* is rich in amino acids, vitamins and minerals particularly iron (Faye et al., 2011).

Hematological parameters

Results in Table 4 show that hemoglobin was not significantly affected by different levels of MLM. Results show a significant increase in WBCs values in the group fed diets containing MLM supplementation in comparison with control. Data show that PCV% was significantly increased with the addition of MLM that may due to numerical increase in RBCs count and significantly increased in WBCs. The increase in the values of PCV indicates a more proper nutrient absorption within the system of the animal. High counts of WBCs enhance adaptability to local environment and disease prevalent conditions (Soetan et al., 2013). The percentage of lymphocytes was significantly decreased, while neutrophils was increased but results were in the normal range.

Immune responses

Results (Fig. 2) indicate that MLM significantly increased antibody titers against SRBCs compared with control group at 7, 14 and 21 days after vaccination. Primary and secondary humoral immune response, the level of specific IgM and IgG, together with the intensity of delayed-type hypersensitivity to sheep erythrocytes were investigated in rabbits fed with MLM load for a month. It is shown that MLM was immunomodulatory. It stimulated numerically IgG and significantly IgM immune response of growing rabbits (Table 4) in comparison with the control group. Our results get along with Olugbemi et al. (2010) who demonstrated that moringa leaves have a beneficial effect on the immune responses and improve intestinal health of broilers. Also, Sudha et al. (2010) showed that moringa leaves methanol extract given orally to mice at doses of 250 and 750 mg/kg stimulated both cellular and humoral immune responses.

Biochemical constituents of blood serum

Results illustrated in Table 4 show that serum cholesterol and LDL levels were significantly reduced as the concentration of MLM meal administered increases. Low-density lipoprotein is a major component of the total cholesterol and is directly related to coronary heart disease as a major atherogenic lipoprotein and hence, appears to be the main target of any lipid lowering agent, such as the moringa leaves, as reflected in our study. Farooq and Rashid (2007) demonstrated that moringa oil from a wild provenance contained phytosterols among which are campesterol, stigmasterol, and β -sitosterol. β -sitosterol which is plant sterol with a structure similar to that of

cholesterol, except for the substitution of an ethyl group at C 24 of its side chain. It could lower cholesterol by lowering plasma concentrations of LDL. This bioactive component of moringa (sitosterol, a bioactive phytoconstituent) may in part be responsible for the hypocholesterolemic effect (Mbikay, 2012). Also, the hypolipidemia of MLM may be due two mechanism actions: HMG-Co-A reductase catalyzes rate limiting process of cholesterol biosynthesis and reduced the absorption of dietary cholesterol and liver cholesterol by biliary secretion (Hassarajani et al., 2007). Results show (Table 4) a significant improved in serum total antioxidant capacity and malondialdehyde in rabbits fed diets containing MLM in comparison with control. Recently, Lamou et al. (2016) reported that aqueous extract of moringa (100, 200, or 400 mg/kg) increased the activity of antioxidant enzymes and decreased the blood concentrations of MAD in rats subjected to forced swimming endurance test.

Result present in Table 4 reveals that serum biochemical indices AST and ALT were not significantly affected, whereas indicating that the treatments have no untoward effect on the health status of the rabbits. These results were in agreement with Ewuola et al. (2015) who showed that unchanged levels of ALT and AST in the serum were an indication of no obvious damage to muscle and organs such as the liver and kidney of rabbits. This result disagree with Adedapo et al. (2009) who found that 400 and 600 mg/kg doses of moringa leaf extract showed a significant increase in the levels of liver enzymes as ALT and AST.

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Table (1): Composition and chemical analysis of moringa and tested diets

Ingredients	Treatments				
	Control	MLM 0.1%	MLM 0.2%	MLM 0.3%	
Corn yellow	19.0	18.9	18.8	18.7	
Wheat bran	11.0	11.0	11.0	11.0	
Barley	17.2	17.2	17.2	17.2	
Berseem hay	33.0	33.0	33.0	33.0	
Soybean meal 44%	15.0	15.0	15.0	15.0	
Molasses	3.0	3.0	3.0	3.0	
Di-Calcium phosphate	1.0	1.0	1.0	1.0	
Lysine	0.1	0.1	0.1	0.1	
Methionine	0.1	0.1	0.1	0.1	
Vitamins premix	0.3	0.3	0.3	0.3	
Nacl	0.3	0.3	0.3	0.3	
Moringa	-	0.1	0.2	0.3	
Total	100	100	100	100	
Chemical analysis determined (DM% basis)					
Chemical analysis%	Dried Moringa leaves	Treatments			
		Control	MIM		
			0.1%	0.2%	0.3%
Organic matter	91.58	91.75	91.75	91.85	91.9
Crude protein	31.62	16.9	17.31	17.40	17.55
Crude fiber	9.35	13.43	13.25	13.27	13.29
Ether Extract	8.42	2.86	2.95	2.97	2.99
NFE*	42.22	58.56	58.24	58.21	58.07
NDF †	35.07	37.75	37.63	37.64	37.66

*Nitrogen free extract (NFE) = (Organic matter) - (Crude protein + Crude fiber + Ether Extract).

† Neutral Detergent Fiber (NDF) = 28.924 + 0.657 × CF%.

Table (2): Overall means of air temperature (AT), relative humidity (RH) and temperature-humidity index (THI).

	AT[°C]			RH%			THI
	Maxi.	Min.	Aver.	Max.	Min.	Aver.	
1- 3 week	29.1	26.4	27.8	62.4	72.7	67.6	26.4
3- 6 week	33.3	29.6	31.5	64.2	76.8	70.5	29.9
Overall mean	31.2	28.0	29.6	63.3	74.8	69.0	28.1

Table (3): Effect of MLM on rabbit's growth performance from 5 to 12 weeks of age (mean±SD)

Characteristics	Control	MLM %			Pvalue
		0.1	0.2	0.3	
Initial body weight, g	705.00±66.89	700.00±69.25	705.83±67.41	706.67±69.21	0.995
Final body weight, g	1960.00 ^b ±125.19	2006.33 ^{ab} ±71.50	2047.50 ^a ±68.14	2048.75 ^a ±59.65	0.044
Daily weight gain, g	26.00±1.82	26.25±2.06	28.00±1.42	28.00±1.72	0.234
Daily feed intake, g	68.42 ^{ab} ±1.42	67.24 ^{bc} ±0.72	65.81 ^c ±0.36	69.68 ^a ±1.7	0.004
Feed conversion ratio	2.64±0.16	2.57±0.18	2.36±0.12	2.49±0.14	0.109

Different letters (a-c) within a row denote significant differences between treatments ($p \leq 0.05$)

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Table (4): Effect of MLM on hematological parameters, serum lipid profile, antioxidant status and liver function of growing rabbits (mean±SD)

Characteristics	Control	MLM %			P value
		0.1	0.2	0.3	
Hematology:					
RBCs*10 ⁶	2.97±0.20	3.08±0.24	3.16±0.20	3.27±0.15	0.09
WBCs*10 ³	5.47 ^c ±0.59	6.17 ^b ±0.37	6.42 ^{ab} ±0.49	6.81 ^a ±0.49	0.001
Hemoglobin, mg/dl	11.72±0.29	11.83±0.20	11.88±0.22	11.85±0.27	0.70
PCV %	38.25 ^b ±1.22	39.85 ^a ±0.71	39.38 ^a ±0.66	39.48 ^a ±0.54	0.01
Eosinophil %	1.47±0.27	1.52±0.20	1.73±0.17	1.58±0.22	0.21
Neutrophils %	35.28 ^c ±1.22	41.60 ^b ±2.69	42.57 ^{ab} ±1.35	44.05 ^a ±1.22	0.001
Lymphocytes %	60.57 ^a ±1.37	54.40 ^b ±2.62	53.32 ^{bc} ±1.22	51.92 ^c ±1.35	0.001
Monocytes %	2.68±0.39	2.48±0.31	2.38±0.12	2.45±0.17	0.22
Immunity traits:					
IgG, mg/dl	211.62±11.39	227.50±19.37	226.33±26.06	224.67±21.36	0.522
IgM, mg/dl	16.94 ^c ±0.22	17.15 ^{bc} ±0.29	17.61 ^{ab} ±0.37	17.78 ^a ±0.59	0.004
Lipid profile:					
Total lipids, mg/dl	174.00±11.83	174.83±13.84	177.33±14.65	177.33±9.50	0.952
Triglycerides, mg/dl	57.49±3.33	58.95±1.91	59.53±1.03	59.63±0.44	0.258
Total cholesterol, mg/dl	100.89 ^a ±4.92	97.57 ^{ab} ±2.18	96.17 ^b ±3.65	93.15 ^b ±2.87	0.01
HDL* mg/dl	52.29±2.19	53.60±1.40	54.59±1.52	54.86±2.16	0.098
LDL* mg/dl	48.61 ^a ±5.04	43.96 ^b ±1.78	41.58 ^{bc} ±4.41	38.29 ^c ±3.09	0.001
Antioxidant status:					
TAC [†] mmol/l	1.44 ^b ±0.15	2.11 ^a ±0.28	2.15 ^a ±0.20	2.28 ^a ±0.29	0.006
MAD [†] nmol/ml	13.80 ^a ±0.47	11.47 ^b ±0.61	11.03 ^{bc} ±0.47	10.44 ^c ±0.64	0.009
Liver functions					
AST [‡] U/L	30.16±2.82	30.20±1.13	30.02±0.76	30.13±1.13	0.998
ALT [‡] U/L	17.87±1.00	18.52±0.69	18.37±0.54	18.59±0.44	0.302

Different letters (a-c) within a row denote significant differences between treatments ($p \leq 0.05$).

*HDL=High density lipoprotein; LDL=Low density lipoprotein.

[†]TAC=Total antioxidant capacity; MAD=Malondialdehyde.

[‡]AST =Aspartate aminotransferase; ALT =Alanine aminotransferase

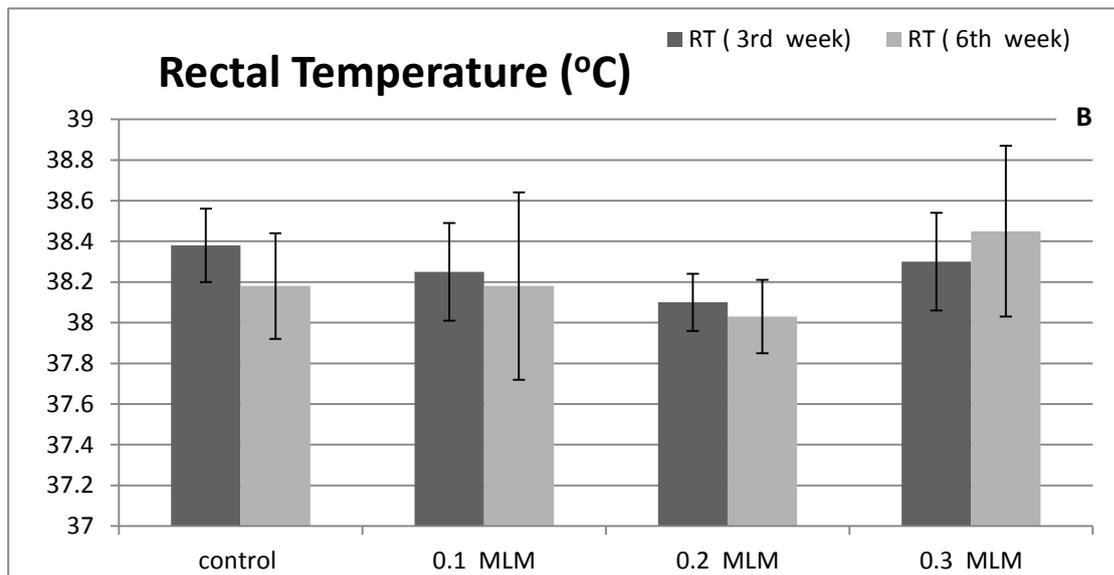
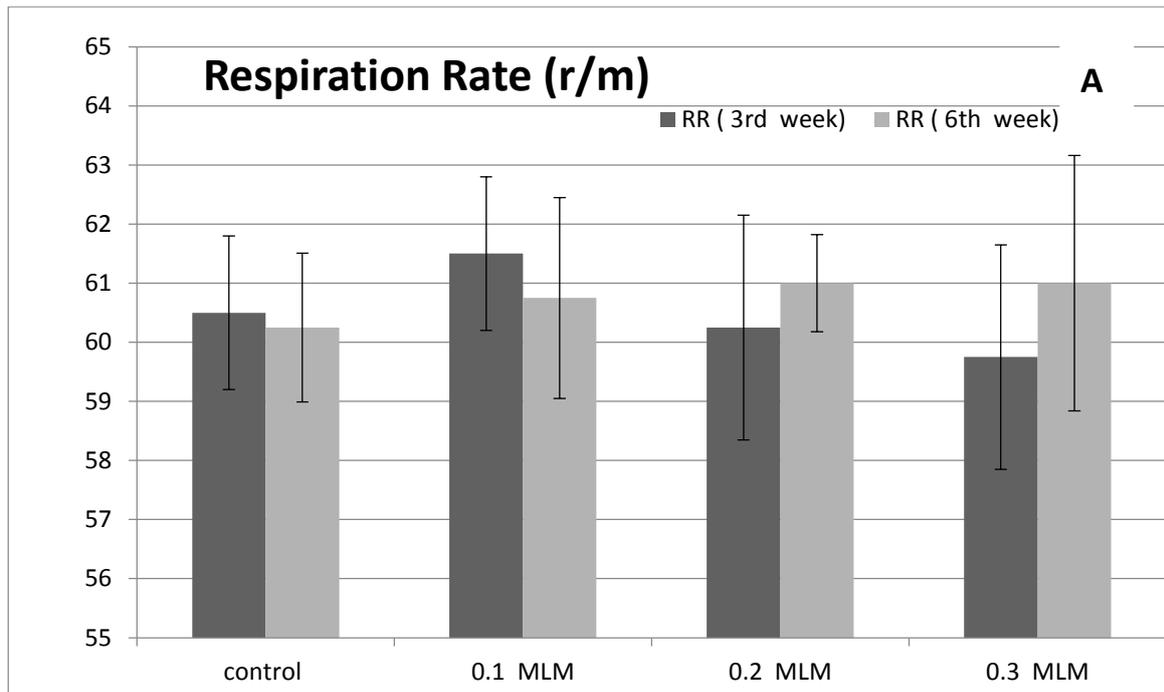


Fig.(1): Effect of MLM on rabbit's (A) respiration rate and (B) rectal temperature during 3rd and 6th week of experiment (mean±SD)

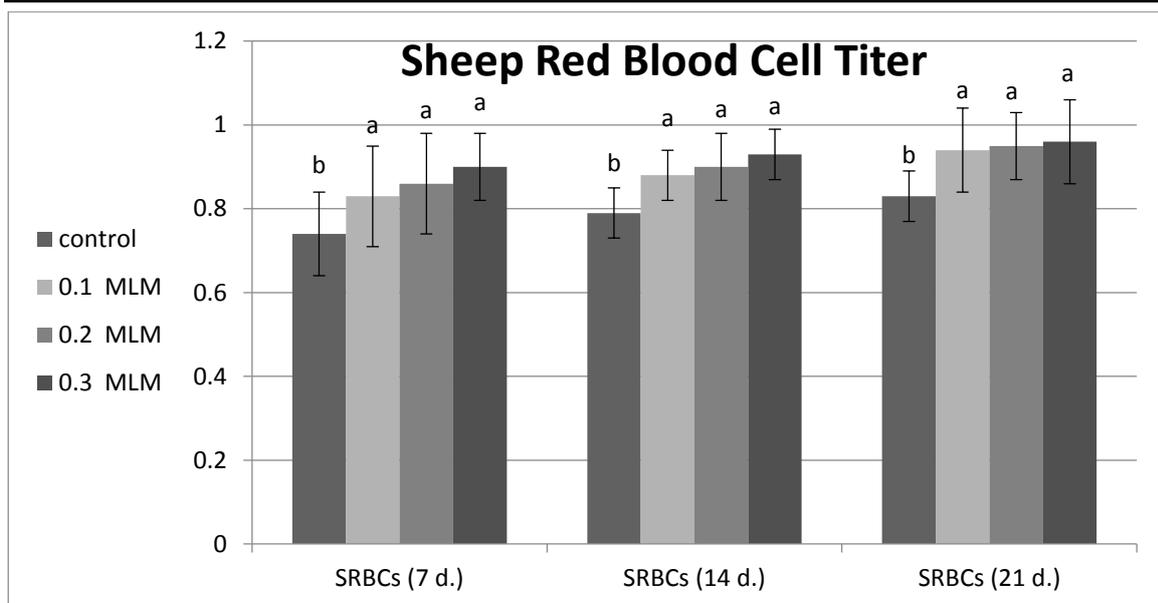


Fig.(2): Effect of MLM on rabbit's responses against sheep red blood cell post 7, 14 and 21 day of vaccination (mean±SD)

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الملخص العربي

تأثير أوراق المورينجا كمضاد طبيعي للأكسدة على الأداء الإنتاجي، دهون الدم والإستجابة المناعية للأرانب تحت الإجهاد الحراري المعتدل

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تهدف الدراسة إلى بحث تأثير مستويات مختلفة من مسحوق أوراق المورينجا على الأداء الإنتاجي والإستجابة المناعية، محتوى الدم من الدهون تحت ظروف الإجهاد الحراري المعتدل. أستخدم في هذه الدراسة 48 أرنب V-line نامى من كلا الجنسين (عمر 5 أسابيع) بمتوسط وزن $68,19 \pm 704,4$ جم. تم توزيع الأرانب عشوائياً على 4 معاملات بكل معاملة 12 أرنب. تم تكوين أربعة علائق تجريبية حيث كانت العليقة الأولى عليقة أساسية بدون أي إضافات (العليقة الضابطة). العليقة الثانية والثالثة والرابعة أضيفت للعليقة الأساسية 0,1، 0,2، 0,3 % من مسحوق أوراق المورينجا المجففة، على التوالي. أوضحت النتائج أن إضافة 0,2 و 0,3 % من أوراق المورينجا أدت إلى زيادة معنوية في وزن الجسم النهائي مقارنة بالمجموعة الضابطة (الكنترول). وأنخفض أستهلاك العليقة معنوياً في مجموعة الأرانب التي تناولت 0,2 % من أوراق المورينجا المجففة مقارنة مع المجموعة الضابطة والمجموعة التي تناولت 0,3 % من أوراق المورينجا خلال الفترة للتجربة. كما أن إضافة أوراق المورينجا المجففة أدت إلى زيادة معنوية في حجم خلايا الدم المتراسة وعدد كرات الدم البيضاء. كما انخفضت نسبة خلايا الدم البيضاء للمفاوية وارتفعت نسبة خلايا الدم البيضاء المتعادلة مقارنة بالكنترول. أدت تغذية الأرانب على مستويات مختلفة من أوراق المورينجا المجففة إلى زيادة معنوية في الإستجابة المناعية للأرانب ضد كريات دم الحمراء للأغنام مقارنة مع المجموعة الضابطة بعد 7، 14، 21 يوم من التحصين. كما أدى استخدام أوراق المورينجا المجففة إلى تنشيط الإستجابة المناعية (الجلوبيولين المناعي IgM) مقارنة مع مجموعة الكنترول. لم تؤثر المعاملات المختلفة على الدهون الكلية، الدهون الثلاثية، الكوليستيرول مرتفع الكثافة بينما تلاحظ إنخفاض معنوى في نسب كلاً من الكوليستيرول، الكوليستيرول منخفض الكثافة والليبيد بيروكسيديز بزيادة مستوى إضافة أوراق المورينجا المجففة في العليقة. كما لوحظ زيادة معنويه في تركيز مجموع المواد المضادة للأكسدة في سيرم دم الأرانب التي تناولت الإضافات من أوراق المورينجا المجففة مقارنة بالمجموعة الضابطة. وخلاصة نتائج البحث أوضحت أن إضافة أوراق المورينجا المجففة في علائق الأرانب النامية له تأثير مفيد على معدل الأداء، دهون الدم، المناعة، الحالة ضد تأكسدية في الأرانب النامية تحت ظروف الإجهاد الحراري المعتدل.