

Effect of dietary moringa (*Moringa oleifera*) and rosemary (*Rosmarinus officinalis*) leaves or their mixture on productive performance, carcass characteristics and antioxidant enzymes of rabbits reared under heat stress conditions

Helal, F. I. S.¹, A.Y. El-Badawi¹, I. El-Wardany², Nematallah G. M. Ali²,
O. M. Aboelazab^{1*}

(1. Animal Production Department, National Research Centre, Dokki, Giza, Egypt;

2. Poultry Production Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt)

Abstract: Sixty growing New Zealand White (NZW) male rabbits aged six weeks, weighed 750.0±5g were used in a feeding experiment lasted 9 weeks. Rabbits were housed in controlled-climatic conditions at a mean ambient temperature 33.1°C and relative humidity 43% to keep rabbits under heat stress conditions. The rabbits were randomly distributed to four equal groups (five replicates each). The 1st group fed the control diet (R1), R2 group fed diet supplemented with 1% moringa leaves, R3 group fed diet supplemented with 1% rosemary leaves, while R4 group fed diet supplemented with a mixture of moringa and rosemary leaves of 0.5% each. Diets and water were provided *ad-libitum* over nine weeks. The results revealed that live body weight, average daily gain and feed conversion ratio were significantly improved with R4 and R2 diets, while daily feed intake was not affected among groups. Apparent digestibility of crude fiber and ether extract were increased ($p \leq 0.05$) with R2 diet. Nitrogen balance was positive for all groups, however improvement ($p \leq 0.05$) was detected with R2 than control. Carcass characteristics were not affected among experimental groups. Hemo-lysatic catalase, glutathione peroxidase and super-oxide dismutase levels were increased ($p \leq 0.01$) with supplemented diets, while malondialdehyde was obviously decreased in the experimental groups compared with the control. It could be concluded that, supplementing diets with 1% moringa dry leaves or mixture of moringa leaves and rosemary leaves by 0.5%+0.5% can be used to improve growth performance, nutrient digestibility and antioxidant status of rabbits reared under heat stress conditions.

Keywords: *Moringa oleifera*, *Rosmarinus officinalis*, rabbits, heat stress, productive performance, antioxidant status

Citation: Helal, F. I. S., A. Y. El-Badawi, I. El-Wardany, N. G. M. Ali, and O. M. Aboelazab. 2017. Effect of dietary moringa (*Moringa oleifera*) and rosemary (*Rosmarinus officinalis*) leaves or their mixture on productive performance, carcass characteristics and antioxidant enzymes of rabbits reared under heat stress conditions. Agricultural Engineering International: CIGR Journal, Special issue: 184–192.

1 Introduction

Global warming and climate change have become major threats for animal production and sustainable livestock production (Gaughan et al., 2009). Rabbits have

few sweat glands, so eliminating the deleterious effects of heat stress becomes difficult to it which leads to poor productive performance.

Biochemical and physiological changes associated with hyperthermia can potentially boost free radicals (reactive oxygen species) formation (Flanagan et al., 1998; Mujahid et al., 2007). Reactive oxygen and nitrogen species (free radicals) are essential to detoxification, chemical signaling and immune function (Anas et al., 2010; Victor et al., 2004). They are continuously produced in the animal body and they are

Received date: 2017-07-25 Accepted date: 2017-12-29

* Corresponding author: Osama M. Aboelazab, Animal Production Department, Agricultural and Biological Research Division, National Research Centre, Egypt - 33 El-Bohouth St., Dokki, Giza, Egypt. P.C.: 12622. Tel: (+20) 1116633693, Fax: (+202) 33370931, Email: os_azab@yahoo.com.

under control by endogenous enzymes such as superoxide dismutase, glutathione peroxidase and catalase. When there is an over-production of these species, an exposure to external oxidant substances or a failure in the defense mechanisms, damage to valuable biomolecules (DNA, lipids, proteins) may occur (Aruoma, 1998), which leads to poor productive performance of animals. Antioxidants are molecules have the ability to neutralize free radicals, mainly by scavenging them and converting them to stable form, thereby preventing or slowing down the deleterious effect of these reactive species to the cellular components. The antioxidant hypothesis says that 'as antioxidants can prevent oxidative damages, increased intakes from the diet will also reduce the risks of chronic diseases' (Stanner et al., 2004).

Several plant extracts and different classes of phytochemicals have been shown to have antioxidant activity (Zheng and Wang, 2001). The search for newer natural antioxidants, especially of plant origin, has ever since has increased. *Moringa oleifera* (The Miracle Tree) is the most widely cultivated species of the genus moringa. *Moringa oleifera* have the ability to prevent effectively, morphological changes and oxidative damage by enhancing the activities of antioxidant enzymes, reducing the intensity of lipid peroxidation and reducing the generation of free radicals (Sreelatha and Padma, 2009). *Moringa oleifera* leaves have multiple antioxidants with high levels, such as phenolic acids (gallic, chlorogenic, ellagic and ferulic acid), glucosinolates and flavonoids (kaempferol, quercetin and rutin) (Mbikay 2012). Furthermore, moringa dry leaves have been reported to be a valuable source of β -carotene (precursor of vit. A) and vitamins (B-complex, C, D and K) (Dorga and Tandon, 1975). Moringa dry leaves have positive effects on hematological measurements of rabbits (Chinwe and Isitua, 2010). Recently, El-Badawi et al. (2014) reported that improvement in nutrients digestibility, dietary N utilization, growth performance and carcass dressing percentage have been recorded for growing rabbits fed diets supplemented with low levels of moringa dry leaves (0.15% and 0.30%).

Rosemary (*Rosmarinus officinalis*) is an herbal plant having needle-like leaves and white, pink, purple, or blue

flowers, native to the Mediterranean region. Through natural antioxidants, rosemary has been clearly accepted as one of the species with the highest antioxidant activity (Peng et al., 2005). The chemical analysis of rosemary showed that it has several types of antioxidants, including flavonoids such as carnosol, carnosic and rosmarinic acid, and volatile oils (Okamura et al., 1994; Angelini et al., 2003).

So, this study was conducted to investigate the effects of supplementing diets with moringa dry leaves, rosemary dry leaves or their mixture (as natural antioxidants sources) on productive performance, carcass characteristics and antioxidant enzymes of rabbits reared under heat stress conditions.

2 Materials and Methods

The present study was carried out at The Rabbits Breeding Farm and The Poultry Physiology Laboratory, Poultry Production Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, and Animal Production Department Laboratory, National Research Centre, Giza, Egypt.

2.1 Plant collection and preparation

Moringa (*Moringa oleifera*) and rosemary (*Rosmarinus officinalis*) leaves were collected from a private commercial farm located in north of Egypt. The leaves of moringa and rosemary were harvested, air-dried under shade until the moisture of collected leaves reached almost 10%, then milled to be ready for the mixing with the other feed ingredients.

2.2 Experimental diets and feeding

Four batches of rabbits feed each of 100 kg were formulated. The control group fed on the basal diet, as stated in Table 1, without any supplement (R1), while moringa and rosemary dry leaves powders were added and thoroughly hand mixed with other feed ingredients at 1% moringa leaves (R2 group), 1% rosemary leaves (R3 group) and a mixture of moringa and rosemary leaves by 0.5%+0.5% each (R4 group). Experimental rations were pelleted at 0.3 cm diameter and the maximum pelleting temperature was not more than 65°C. A feeding trial lasted 63 days was conducted on sixty male growing New Zealand White rabbits (NZW) aged six weeks with an

average body weight of 750.0 ± 5 g. Rabbits were randomly distributed by weight in four equal groups. The feed was offered *ad-libitum* and water was freely choice during the experimental period. Experimental rabbits were weekly weighed and recorded.

Table 1 Composition of the basal diet

Ingredients	%
Alfalfa hay	32.00
Soybean meal (44%)	21.00
Yellow corn	16.00
Barley	16.00
Wheat bran	9.20
Cane-molasses	3.00
Limestone	0.90
Di-Ca-P	0.90
Premix*	0.30
NaCl (salt)	0.30
DL-Methionine	0.20
Mold Guard	0.10
Anti-coccidian	0.10
Total	100

Note: * Each 3 kg contains: vit A 12000000 IU, vit D₃ 2500000 IU, vit E 10 g, vit K₃ 2 g, vit B₁ 1 g, vit B₂ 5 g, vit B₆ 1.5 g, vit B₁₂ 0.01 g, Niacin 30 g, Folic 1 g, Biotin 0.05 g, Pantothenic acid 10 g, Copper 10 g, Iodine 1 g, Selenium 0.1 g, Iron 30 g, Manganese 60 g, Zinc 50 g, Cobalt 0.1g.

2.3 Chemical analysis

Chemical analysis of the basal diet, moringa dry leaves, rosemary dry leaves (Table 2) and slaughtered animals (Table 8) were determined for dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and ash according to the standard methods of AOAC (2012). Nitrogen free extract (NFE) was calculated by difference.

Table 2 Chemical composition of basal diet, moringa dry leaves and rosemary dry leaves

Item	Basal diet	Moringa dry leaves	Rosemary dry leaves
Moisture, %	9.04	7.92	7.69
DM composition, %			
Organic matter (OM)	89.47	87.34	91.76
Crude protein (CP)	16.81	19.52	4.10
Crude fiber (CF)	8.42	7.23	18.97
Ether extract (EE)	3.79	7.51	16.32
Nitrogen free extract (NFE)	60.45	53.08	52.37
Ash	10.53	12.66	8.24

2.4 Total antioxidant determination

Total antioxidant capacity (TAC) of the basal diet, moringa and rosemary dry leaves (Table 3) were determined by the method described by Koracevic et al. (2001). The extraction of antioxidants from the samples

was performed by using 80% methanol at 35°C for 24 hours as stated by Cai et al. (2004) and the ratio between sample and solvent was 1 g sample: 20 mL solvent. The calculated values of TAC for the four experimental rations were 0.396, 0.406, 0.407 and 0.407 mmol L⁻¹ for R1, R2, R3 and R4 groups, respectively.

Table 3 Total antioxidant capacity of basal diet, moringa leaves and rosemary leaves

Item	Basal diet	Moringa leaves	Rosemary leaves
Total antioxidant capacity, mmol/l	0.396	1.022	1.096

2.5 Climatic management

A semi-climatic concrete chamber of 3×3×2.5 m dimensions fitted with controlled speed suction fan, electrical heaters, thermometer and hygrometer were used to keep the ambient temperature at $33.1 \pm 0.5^\circ\text{C}$ and relative humidity at $43\% \pm 3\%$ for the whole experimental period (Table 4).

Table 4 Average of temperature (°C), relative humidity (%) and temperature-humidity index (THI) through experimental period

Period	Temperature	Relative humidity	THI
1 st week	33.2	41	29.76
2 nd week	32.8	44	29.61
3 rd week	33.5	42	30.07
4 th week	32.8	46	29.72
5 th week	32.8	44	29.61
6 th week	33.4	42	29.98
7 th week	33.5	42	30.07
8 th week	32.7	44	29.52
9 th week	32.9	46	29.80
Overall	33.1	43	29.80

The relationship between temperature and relative humidity was termed temperature-humidity index (THI). This parameter indicates the presence of heat stress or not. THI had been modified by Marai et al. (2001) to be suitable for rabbits (as small animals) and was calculated as

$$\text{THI} = \text{db } ^\circ\text{C} - [(0.31 - 0.31\text{RH})(\text{db } ^\circ\text{C} - 14.4)]$$

where, db °C= dry bulb temperature in degrees Celsius and RH= relative humidity/100.

The values obtained are then classified as follows: <27.8= absence of heat stress, 27.8–28.9= moderate heat stress, 28.9–30.0= severe heat stress and 30.0 and more= very severe heat stress. In the present study, overall averages of temperature, relative humidity and THI were

33.1°C, 43% and 29.80 respectively, indicated that the rabbits were reared under severe heat stress conditions.

2.6 Growth performance

The individual live body weight of rabbits was recorded at weekly intervals up to fifteen weeks of age. Also, the body weight gain (BWG) and feed intake were recorded at weekly intervals and the feed conversion ratio (FCR) was calculated as feed consumed per unit BWG.

2.7 Digestibility trial

At the end of the experimental period, three rabbits were randomly taken from each group to evaluate nutrients digestion coefficient and dietary nitrogen utilization. A preliminary period of four days (for adaptation) was followed by three days for fecal and urine collection. Feces of each rabbit were collected quantitatively once daily before feeding, and the daily feces of each rabbit were oven dried at 60°C overnight. Urine of each animal was daily collected and composite sample of 10% of the daily urine of each rabbit was kept in glass bottle until nitrogen determination. Total digestible nutrients (TDN) and digestible crude protein (DCP) were determined according to Cheeke et al. (1987).

2.8 Carcass characteristics

After termination of the feeding experiment, three representative rabbits were randomly taken from each group and fasted for 12 hrs. Rabbits were hand slaughtered, and the slaughtered animals were de-skinned, dressed out and the hot carcass including head was weighed and recorded. Edible offals (liver, heart, spleen and kidneys), non-edible offals [lungs & trachea, clean empty gastro-intestinal tract (G.I.T.) and testicles] and trimmings (fur, four legs, blood and G.I.T. contents) were separately weighed and recorded. The empty body weight was calculated by deducting the G.I.T. contents weight from fasting body weight. The boneless meat of each slaughtered rabbit was minced then dried at 60°C for 48 h. Dried minced meat was chemically analyzed according to AOAC (2012).

2.9 Blood antioxidant enzymes

Blood samples were taken at the end of the experimental period from animals at slaughtering time. Three samples per each treatment group were collected in heparinized tubes, centrifuged under cooling conditions.

The hematocrit remaining after harvesting the plasma from the previous step was taken to assay catalase, glutathione peroxidase (GSH-Px), super-oxide dismutase (SOD) and malondialdehyde (MDA) using commercial kits purchased from (Bio-Med Diagnostics, Egypt. Co. for Biotechnology). The GSH-Px was determined according to the method described by Paglia and Valentine (1967). SOD was determined according to Nishikimi et al. (1972), while catalase was determined according to the method stated by Fossati et al. (1980). The determination of MDA levels was based on a colorimetric method as described by Ohkawa et al. (1979).

2.10 Statistical analysis

Collected data were subjected to one-way analysis of variance by using the General Linear Model Procedure (GLM) of the Statistical Analysis System (SAS, 1998). Differences among treatment means were detected by using Duncan's multiple range test (Duncan, 1955).

3 Results and discussion

3.1 Growth Performance

Average daily feed intake of rabbits fed experimental rations (Table 5) showed no significant differences among the experimental groups, while live body weight, average daily weight gain and feed conversion ratio were significantly improved for rabbits fed diets supplemented with the mixture of moringa and rosemary dry leaves by 0.5%+0.5% of each (R4) compared with the control group (R1). Also, the R2 group showed a significant increase in the final body weight, but no effect on FCR, compared with the control. The present results are consistent with the findings of Hassan et al. (2016) who reported that addition of *Moringa oleifera* leaves meal up to 0.3% of broiler diets improved growth performance of broiler chickens reared under heat stress conditions. In the same trend, El-Badawi et al. (2014) reported that moringa supplemented diets had a positive effect on feed conversion ratio and average daily gain of rabbits fed 0.15 and 0.30% moringa supplemented rations. Also, Banjo (2012) reported that inclusion of dietary moringa significantly ($P<0.05$) enhanced body weight gain of broiler chickens at 2% level of inclusion but did not affect feed intake or feed conversion ratio.

Table 5 Growth performance of NZW rabbits in experimental groups

Item	Experimental groups				MSE	Significance
	R1	R2	R3	R4		
Initial body weight, g	755	752	755	754	6.36	NS
Final body weight, g	1962 ^c	2020 ^b	1908 ^c	2154 ^a	27.91	*
Average daily weight gain, g	19.2 ^{bc}	20.1 ^b	18.3 ^c	22.2 ^a	0.52	*
Average daily feed intake, g	71.1	73.2	68.7	69.7	4.48	NS
Feed conversion ratio	3.71 ^a	3.63 ^a	3.75 ^a	3.14 ^b	0.19	*

Note: a, b and c: Means in the same row having different superscripts are significantly different. MSE=Mean of standard errors, NS= non-significant, * $P \leq 0.05$. R1=control; R2= 1% moringa; R3= 1% rosemary; R4= 0.5% moringa + 0.5% rosemary.

Concerning rosemary effect, the present results are in agreement with Erdelyi et al. (2008) who showed that rosemary oil supplementation to growing rabbit diets by level of 0.15% showed some beneficial, but not statically significant effects. On the contrast, Ghazalah and Ali (2008) reported that broiler chicks fed 0.5% rosemary leaves meal exhibited higher body weights, greater weight gain and better feed conversion compared with chicks fed 1% or 2% rosemary leaves meal.

The response of rabbit's body weight to rations supplemented with moringa or rosemary leaves was observed during the last four weeks of feeding period (Figure 1). Body weight development curve showed a clear positive effect of feeding R4 and R2, while there was a slight decrease (non-significant) in body weight with separate rosemary supplementation (R3) especially in the last three weeks of the feeding period.

Improvement of live body weight occurred in rabbits fed moringa leaves separately or in combination with rosemary leaves in the present study might be due to the high antioxidant content of moringa leaves (1.022 mmol L⁻¹ as mentioned previously), furthermore, high portion of these antioxidants is vitamins C and A (USDA, 2016) which act as powerful antioxidants and have the ability to eliminate the adverse effects of excess free radicals resulted from heat stress. While rosemary leaves also have antioxidant properties (1.096 mmol L⁻¹), as it has high contents of flavonoids (Zeghad and Merghem, 2016), but it could not have the ability to compensate the negative effects of heat stress on rabbits' growth performance when supplemented separately, indicating

that flavonoids are low in antioxidant power compared with vitamins A and C. The most improvement of live body weight that observed in rabbits fed the mixture of moringa and rosemary leaves (R4 group) declared that there is a synergism effect between the two supplements at 0.5% level in the face of the negative effects of heat stress conditions.

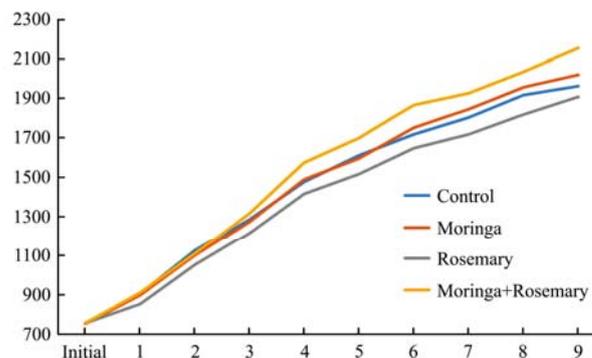


Figure 1 Live body weight development of rabbits in experimental groups

3.2 Nutrient digestibility and nitrogen utilization

Nutrients digestibility coefficients and nitrogen utilization of the rabbits as affected by feeding treatments are shown in Table 6. The incorporation of moringa leaves, rosemary leaves or their combination in rabbit diets resulted in non-significant differences in the digestibility coefficients of dry matter (DM), organic matter (OM), crude protein (CP) and nitrogen free extract (NFE), while there was an increase in crude fiber digestibility in the experimental groups compared with the control group. However, there was an improvement in ether extract digestibility in rabbits fed moringa or rosemary leaves separately compared with those fed the mixture of the two supplements. Furthermore, dietary nitrogen intake, digestible nitrogen and absolute nitrogen balance increased among experimental groups compared with the control, while non-significant ($P > 0.05$) diet effects were observed for faecal nitrogen, urinary nitrogen and total nitrogen excretion among treatments.

The results obtained are in disagreement with El-Badawi et al. (2014) who reported that OM and CP digestibility's in rabbits were significantly higher in animals fed moringa supplemented rations, but the same study showed that moringa dry leaves has positive effects on digestible nitrogen and nitrogen balance. Similar studies stated the non-significant effect of supplementing

dietary *Moringa oleifera* leaf meal on nutrient digestibility, Nkukwana et al. (2014), as the results of the present study. Furthermore, essential oil from rosemary has been shown to improve apparent whole-tract and ileal digestibility in rabbits (Hernández et al., 2004), which has not been observed in the present study except for crude fiber digestibility.

Table 6 Nutrients digestibility and nitrogen utilization of NZW rabbits in experimental groups

Item	Experimental groups				MSE	Significance
	R1	R2	R3	R4		
Digestion coefficients, %						
Dry matter	62.92	64.49	63.61	66.60	1.48	NS
Organic matter	64.03	67.27	64.53	66.01	3.13	NS
Crude protein	73.09	74.73	70.49	74.59	2.39	NS
Crude fiber	47.15 ^b	52.66 ^a	53.68 ^a	53.26 ^a	2.51	*
Ether extract	56.49 ^{ab}	63.10 ^a	61.53 ^a	51.91 ^b	3.88	*
Nitrogen free extract	65.88	69.79	68.70	68.41	3.95	NS
Nutritive values, %						
Total digestible nutrients	59.65	63.66	61.29	63.87	2.60	NS
Digestible crude protein	11.61 ^a	11.67 ^a	10.56 ^b	11.87 ^a	0.43	*
Nitrogen utilization, g						
Nitrogen intake (NI)	2.41 ^b	3.00 ^a	2.82 ^{ab}	2.57 ^{ab}	0.25	*
Fecal nitrogen	0.65	0.76	0.83	0.65	0.09	NS
Digestible nitrogen	1.76 ^b	2.25 ^a	1.99 ^{ab}	1.92 ^{ab}	0.21	*
Urinary nitrogen	0.23	0.20	0.24	0.22	0.03	NS
Total nitrogen excretion	0.88	0.96	1.07	0.87	0.10	NS
Nitrogen balance (NB)	1.53 ^b	2.05 ^a	1.75 ^{ab}	1.70 ^{ab}	0.21	*
NB/NI, %	63.68	68.01	62.10	65.87	2.89	NS

Note: a and b: Means in the same row having different superscripts are significantly different. MSE=Mean of standard errors, NS= non-significant, * $P \leq 0.05$. R1=control; R2= 1% moringa; R3= 1% rosemary; R4= 0.5% moringa + 0.5% rosemary.

As reported by Gidenne (2000), the rabbit has a low utilization of the fibrous fraction due to the rapid passage of feed through the gastrointestinal tract. Also, it is well-known that heat stress causes excess free radicals production, which in turn can increase the lipid oxidation and produce lipid peroxides (Andrea et al., 2004), which could damage the antioxidative capability of animals. The last impact could be terminated or delayed by dietary addition of natural antioxidants presented in moringa and rosemary leaves which explains the observed increment of crude fiber and ether extract digestibilities in the present study. Although the high absolute nitrogen balance (NI) observed in experimental groups, but the non-significant relative NI to nitrogen intake can illustrate this observation.

The high nitrogen intake and digestibility values of moringa and rosemary treatments may be due to the low anti-nutritional compounds of *Moringa oleifera* along with their highly digestible nature which reported by Nuhu (2010). Besides, supply of rosemary to diets increased DM intake and subsequently nitrogen intake (Smeti et al., 2015), which can be related to the ability of terpenes compounds, presented in rosemary leaves, to improve the fermentative processes in the cecum, inhibiting protein utilization by endogenous microorganisms (Chiofalo et al., 2010).

3.3 Carcass characteristics and meat composition

Carcass characteristics and the chemical composition of the rabbit's meat are shown in Tables 7 and 8, respectively. Slaughter weight, empty body weight, dressing percentage, total edible offals, non-edible offals and trimmings were not affected significantly ($P > 0.05$) for rabbits fed experimental diets. Also, protein or fat contents of meat were not affected among experimental treatments. The present results are in close agreement with similar findings by many investigators (Nuhu, 2010; Abbas and Ahmed, 2012) who reported that the slaughter weight, hot carcass weight, dressed weight and dressing percentage were not affected by supplementing the diets with *Moringa oleifera* leaf meal in growing rabbits and broiler chickens, respectively.

Moreover, Ciftci et al. (2013) stated that rosemary leaves have no effects on hot and cold carcass percentages of Japanese quail reared under heat stress conditions. On the contrary, El-Tazi (2014) and El-Badawi et al. (2014) reported that inclusion of moringa leaves in the diets of broilers and rabbits, respectively, improved hot and cold carcass weight, dressing percentage and lean meat yield. Also, Ghazalah and Ali (2008); Tollba (2010) mentioned that the addition of rosemary leaves to broiler diets have positive effects on carcass weight, carcass percentage, giblets and dressing percentages.

Although the powerful effects of rosemary and moringa on enhancing the meat quality properties that is discussed by many investigators (Debersac et al., 2001; Yesilbag et al., 2011; Kahraman et al., 2015; Dany et al., 2016), but these impacts did not extend to affect carcass characteristics or meat chemical composition.

Table 7 Carcass characteristics of NZW rabbits in experimental groups

Item	Experimental groups				MSE	Significance
	R1	R2	R3	R4		
Live body weight (LBW), g	2033	2092	2003	2000	268	NS
Empty body weight (EBW), g	1850	1918	1834	1822	264	NS
Hot carcass weight, g	1120	1158	1098	1097	180	NS
Dressing, %						
Carcass % (LBW)	55.11	55.25	54.58	54.66	2	NS
Carcass % (EBW)	60.56	60.28	59.68	60.08	1.8	NS
Edible offals (giblets) weight, g						
Heart	5.76	5.13	5.72	4.45	1.14	NS
Liver	52.45	54.13	47.97	56.91	11.11	NS
Defatted kidneys	11.58	10.69	11.65	11.04	1.71	NS
Spleen	1.24	1.27	1.04	1.27	0.55	NS
Head and ears	164.3	163.3	168.3	150	23.03	NS
Total, %	12.71	12.26	12.82	12.29	0.51	NS
Non-edible offals, g						
Lungs and trachea	9.7	10.2	10.8	13	2.62	NS
Empty GIT	122	116	113	119	10.8	NS
Testicles	7.3	9.7	7.9	8.2	1.77	NS
Total, %	7.5	7.11	7.26	7.8	0.85	NS
Trimmings, g						
Fur	193	218	213	175	32.2	NS
Four legs	57	53	60	51	9.3	NS
Blood	53	68	58	53	8.8	NS
GIT contents	183	174	169	178	11.8	NS
Total, %	26.31	26.94	27.55	25.21	1.8	NS

Note: MSE=Mean of standard errors, NS= non-significant. R1=control; R2= 1% moringa; R3= 1% rosemary; R4= 0.5% moringa + 0.5% rosemary.

Table 8 Body composition (%) of slaughtered NZW rabbits in experimental groups (on fresh basis)

Item	Experimental groups				MSE	Significance
	R1	R2	R3	R4		
Water content	69.39	67.98	68.83	68.69	2.42	NS
Crude protein	22.19	21.31	21.86	22.06	1.09	NS
Extracted fat	6.43	8.83	7.39	7.39	1.74	NS
Ash	1.45	1.32	1.31	1.33	0.15	NS

Note: MSE=Mean of standard errors, NS= non-significant. R1=control; R2= 1% moringa; R3= 1% rosemary; R4= 0.5% moringa + 0.5% rosemary.

Activities of antioxidant enzymes: Results of hemo-lysatic catalase, glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and malondialdehyde (MDA) as affected by experimental feed supplements are presented in Table 9. The major three antioxidant enzymes were significantly increased ($P<0.01$), while MDA decreased simultaneously in animals received dietary supplements of moringa and rosemary leaves compared with the control group. The most

improvements were noticed in R4 and R2 groups which received moringa supplements even in combination with rosemary or separately.

Table 9 Antioxidant enzymes and malondialdehyde of NZW rabbits in experimental groups.

Item	Experimental groups				MSE	Significance
	R1	R2	R3	R4		
Catalase	50.39 ^b	64.57 ^a	61.02 ^a	60.51 ^a	2.43	*
Glutathione peroxidase	55.58 ^c	94.01 ^a	65.21 ^b	90.11 ^a	4.07	*
Super-oxide dismutase	21.99 ^c	25.82 ^b	25.13 ^b	28.96 ^a	1.43	*
Malondialdehyde	66.27 ^a	38.79 ^b	31.33 ^c	29.05 ^c	3.88	*

Note: a, b and c: Means in the same row having different superscripts are significantly different. MSE=Mean of standard errors, * $P\leq 0.01$. R1=control; R2= 1% moringa; R3= 1% rosemary; R4= 0.5% moringa + 0.5% rosemary.

The present results are in close agreement with similar findings by many investigators (Sutalangka et al., 2013; Oseni and Idowu, 2014; Oparinde and Atiba, 2014) who reported that moringa leaves or extract of *Moringa oleifera* leaf increased GSH, GSH-Px, catalase and inhibition percentage of SOD but reduced MDA concentrations in rats. Also, Yesilbag et al. (2011); Ozcelik et al. (2014) reported that supplementing diets with rosemary leaves or rosemary oil reduced serum MDA levels and improved SOD and GSH-Px activity in heat stressed broiler or Japanese quail, respectively.

In general, heat stress causes a negative change in antioxidant status of rabbits inducing oxidative stress, which causes an increase in MDA and the decrease of endogenous antioxidant enzymes as noticed in the control group (R1). Moreover, the significant increase in the three major antioxidant enzymes (catalase, GSH-Px and SOD) may be attributed to the high effect of polyphenols, vitamins C and A of moringa leaves. On the same side, the potent antioxidant properties of rosemary leaves have been mainly attributed to its major diterpenes, carnosol and carnosic acid, as well as to the essential oil components (Ngo et al., 2011).

4 Conclusion

From the present results, it could be concluded that adding moringa leaves by 1% separately or in combination with rosemary leaves by level 0.5% +0.5% of each could improve the growth performance and endogenous antioxidant status of growing rabbits reared under heat stress conditions.

References

- Abbas, T. E., and M. E. Ahmed. 2012. Use of *Moringa oleifera* seeds in broilers diet and its effects on the performance and carcass characteristics. *International Journal of Applied Poultry Research*, 1(1): 1–4.
- Anas. A. A., W. J. Wiersinga, A. F. de Vos, and D. P. T. Van. 2010. Recent insights into the pathogenesis of bacterial sepsis. *Netherlands Journal of Medicine*, 68(4): 147–152.
- Andrea. S., H. Frank, and K. Eder. 2004. Thermally oxidized dietary fat upregulates the expression of target genes of PPAR α in rat liver. *Journal of Nutrition*, 134(6): 1375–1383.
- Angelini, L. G., G. Carpanese, P. L. Cioni, T. Morelli, M. Macchia, and G. Flamini. 2003. Essential oils from Mediterranean lamiaceae as weed germination inhibitors. *Journal of Agriculture and Food Chemistry*, 51(21): 6158–6164.
- AOAC. 2012. *Official Methods of Analysis*, 19th ed. Association of Official Analytical Chemists, Washington, DC, USA.
- Aruoma, I. O. 1998. Free radicals, oxidative stress, and antioxidants in human health and disease. *Journal of the American Oil Chemists Society*, 75(2): 199–212.
- Banjo, O. S. 2012. Growth and performance as affected by inclusion of *Moringa oleifera* leaf meal in broiler chicks diet. *Journal of Biology Agriculture and Healthcare*, 2(9): 35–38.
- Cai, Y., Q. Luo, M. Sun, and H. Corke. 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Science*, 74(17): 2157–2184.
- Cheeke, P. R., N. M. Patton, S. D. Lukefahr, and I. J. McNitt. 1987. *Rabbit Production*, 6th ed. Interstate Printers and Publishers Inc. Danville, IL, USA.
- Chinwe, C., and N. Isitua. 2010. Studies on the hematological impact of *Moringa oleifera* in rabbits. A poster presented at 2nd Int. Conf. on Appl. Biotech., Khartoum, Sudan.
- Chiofalo, B., E. R. Riolo, G. Fasciana, L. Liotta, and V. Chiofalo. 2010. Organic management of dietary rosemary extract in dairy sheep: effects on milk quality and clotting properties. *Veterinary Research Communications*, 34: S197–S201.
- Ciftci, M., U. G. Simsek, M. A. Azman, I. H. Cerci, and F. Tonbak. 2013. The effects of dietary rosemary (*Rosmarinus officinalis* L.) oil supplementation on performance, carcass traits and some blood parameters of Japanese quail under heat stressed condition. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, 19(4): 595–599.
- Dany, D. C., O. O. Jorge, S. V. Ángell, S. D. Enrique, P. R. Valentín, M. H. Victor, and S. F. Luis. 2016. Effect of *Moringa oleifera* meal inclusion on meat quality from the Mexican hairless pig. *ARNP Journal of Agricultural and Biological Science*, 11(4): 131–141.
- Debersac, P., M. Vernevaut, M. Amiot, M. Suschetet, and M. Siess. 2001. Effects of a water-soluble extract of rosemary and its purified component rosmarinic acid on xenobiotic-metabolizing enzymes in rat liver. *Food & Chemical Toxicology*, 39(2): 109–117.
- Dorga, P. D. S., and S. Tandon. 1975. Vitamin content in moringa. *International Journal of Current Science*, 44: 30–31.
- Duncan, D. B. 1955. Multiple rang and multiple F–test. *Biometrics*, 11: 1–42.
- El-Badawi, A. Y., H. A. A. Omer, A. A. Abedo, and M. H. M. Yacout. 2014. Response of growing New Zealand white rabbits to rations supplemented with different levels of *Moringa oleifera* dry leaves. *Global Veterinaria*, 12(4): 573–582.
- El-Tazi, S. M. A. 2014. Effect of feeding different levels of *Moringa oleifera* leaf meal on the performance and carcass quality of broiler chicks. *Journal of International Medical Research*, 3(5): 147–151.
- Erdelyi, M., Z. Matics, Z. Gerencsér, Z. Princz, Z. Szendro, M. Mézes. 2008. Study of the effect of rosemary (*Rosmarinus officinalis*) and garlic (*Allium sativum*) essential oils on the performance of rabbit. Proc. 9th World Rabbit Congress – Verona, Italy, pp: 649–653.
- Flanagan, S. W., P. L. Moseley, and G. R. Buettner. 1998. Increased flux of free radicals in cells subjected to hyperthermia: Detection by electron paramagnetic resonance spin trapping. *FEBS Letters*, 431(2): 285–286.
- Fossati, P., L. Prencipe, and G. Berti. 1980. Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. *Clinical Chemistry*, 26(2): 227–231.
- Gaughan, J. B., K. L. Ebi, I. Burton, and G. R. McGregor. 2009. Response of domestic animals to climate challenges. In *Biometeorology for Adaptation to Climate Variability and Change*. I. Burton, K. L. Ebi, eds. Berlin: Germany: Springer, 1: 131–170.
- Ghazalah, A. A., and A. M. Ali. 2008. Rosemary leaves as a dietary supplement for growth in broiler chickens. *International Journal of Poultry Science*, 7(3): 234–239.
- Gidenne, T. 2000. Recent advances in rabbit nutrition: emphasis on fibre requirements. A review. *World Rabbit Science*, 8: 23–32.
- Hassan, H. M. A., M. M. El-Moniary, Y. Hamouda, E. F. El-Daly, A. W. Youssef and N. A. A. El-Azeem. 2016. Effect of different levels of *Moringa oleifera* leaves meal on productive performance, carcass characteristics and some blood parameters of broiler chicks reared under heat stress conditions. *Asian Journal of Animal and Veterinary Advances*, 11: 60–66.
- Hernández, P., S. Aliaga, M. Pla, and A. Blasco. 2004. The effect of selection for growth rate and slaughter age on carcass composition and meat quality traits in rabbits. *Journal of Animal Science*, 82: 3138–3143.

- Kahraman, T., G. Issa, E. B. Bingol, B. B. Kahraman, and E. Dumen. 2015. Effect of rosemary essential oil and modified-atmosphere packaging (MAP) on meat quality and survival of pathogens in poultry fillets. *Brazilian Journal of Microbiology*, 46(2): 591–599.
- Koracevic, D., G. Koracevic, V. Djordjevic, S. Andrejevic, and V. Cosic. 2001. Method for the measurement of antioxidant activity in human fluids. *American Journal of Clinical Pathology*, 54(5): 356–361.
- Marai, I. F. M., M. S. Ayyat, and U. M. A. El-Monem. 2001. Growth performance and reproductive traits at first parity of New Zealand white female rabbits as affected by heat stress and its alleviation under Egyptian conditions. *Tropical Animal Health & Production*, 33(6): 451–462.
- Mbikay, M. 2012. Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: a review. *Frontiers Pharmacol*, vol. 3, Article 24.
- Mujahid, A., N. R. Pumford, W. Bottj, K. Nakagawa, T. Miyazawa, Y. Akiba and M. Toyomizu. 2007. Mitochondrial oxidative damage in chicken skeletal muscle induced by acute heat stress. *Journal of Poul Poultry Science*, 44: 439–445.
- Ngo, S. N., D. B. Williams, and R. J. Head. 2011. Rosemary and cancer prevention: preclinical perspectives. *Critical Reviews in Food Science and Nutrition*, 51(10): 946–954.
- Nishikimi, M., N. Appaji, and K. Yagi. 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical and Biophysical Research Communications*, 46(2): 849–854.
- Nkukwana, T. T., V. Muchenje, E. Pieterse; P. J. Masika, T. P. Mabusela, L. C. Hoffman, and K. Dzama. 2014. Effect of *Moringa oleifera* leaf meal on growth performance, apparent digestibility, digestive organ size and carcass yield in broiler chickens. *Livestock Science*, 161: 139–146.
- Nuhu, F. 2010. Effect of moringa leaf meal (MOLM) on nutrient digestibility, growth, carcass and blood indices of weaner rabbits. M.Sc., Fac. Agric. Natural Res., Kwame Nkrumah Univ. Sci. Tech., Kumasi, Ghana.
- Ohkawa, H., N. Ohishi, and K. Yagi. 1979. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. *Annals Biochemistry*, 95(2): 351–358.
- Okamura, N., H. Haraguchi, K. Hashimoto, and A. Yagi. 1994. Flavonoids in *Rosmarinus officinalis* leaves. *Phytochemistry*, 37(5): 1463–1466.
- Oparinde, D. P., and A. S. Atiba. 2014. *Moringa oleifera* leaf prevents oxidative stress in Wistar rats. *European Journal of Medicinal Plants*, 4(9): 1150–1157.
- Oseni, O. A., and A. S. K. Idowu. 2014. Inhibitory activity of aqueous extracts of horseradiash *Moringa oleifera* (lam) and nutmeg *Myristica fragrans* (houitt) on oxidative stress in alloxan induced diabetic male Wistar Albino rats. *American Journal of Biochemistry and Biotechnology*, 4(2): 64–75.
- Ozcelik, M., U. G. Simsek, S. Ceribasi, and M. Ciftci. 2014. Effects of different doses of rosemary oil (*Rosmarinus officinalis* L.) on oxidative stress and apoptosis of liver of heat stressed quails. *European Poultry Science*, 78. DOI: 10.1399/eps.2014.32.
- Paglia, D. E., and W. N. Valentine. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine*, 70(1): 158–69.
- Peng, Y., J. Yuan, F. Liu, and J. Ye. 2005. Determination of active components in rosemary by capillary electrophoresis with electrochemical detection. *Journal of Pharmaceutical and Biomedical Analysis*, 39(3-4): 431–437.
- SAS. 1994. *SAS/STAT User's Guide: Statistics*. Ver. 6.04, 4th ed. SAS Institute Inc., Cary, NC.
- Smeti, S., M. Joy, H. Hajjl, J. L. Alabart, F. Munoz, M. Mahouachi, and N. Atti. 2015. Effects of *Rosmarinus officinalis* L. essential oils supplementation on digestion, colostrum production of dairy ewes and lamb mortality and growth. *Journal Animal Science*, 86: 679–688.
- Sreelatha, S., and P. R. Padma. 2009. Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. *Journal of Plant Food Human Nutrition*, 64(4): 303–311.
- Stanner, S. A., J. Hughes, C. N. Kelly, and J. Buttriss. 2004. A review of the epidemiological evidence for the antioxidant hypothesis. *Public Health Nutrition*, 7(3): 407–22.
- Sutalangka, B., J. Wattanathorn, S. Muchimapura, and W. Thukham-mee. 2013. *Moringa oleifera* mitigates memory impairment and neurodegeneration in animal model of age-related dementia. *Oxidative Medicine and Cellular Longevity*, (1):695936.
- Tollba, A. A. H. 2010. Reduction of broilers intestinal pathogenic microflora under normal or stressed condition. *Egyptian Poultry Science Association*, 30(1): 249–270.
- USDA. 2016. Full Report (All Nutrients) 11222, Drumstick leaves, raw. National Nutrient Database for Standard Reference, Release 28 slightly revised May, 2016.
- Victor, V. M., M. Rocha, and L. F. M. De. 2004. Immune cells: free radicals and antioxidants in sepsis. *International Immunopharmacology*, 4: 327–347.
- Yesilbag, D., M. Eren, H. Agel, A. Kovanlikaya, and F. Balci. 2011. Effects of dietary rosemary, rosemary volatile oil and vitamin E on broiler performance, meat quality and serum SOD activity. *British Poultry Science*, 52(4): 472–482.
- Zeghad, N., and R. Merghem. 2016. Antioxidant activity of flavonoids isolated from *Rosmarinus officinalis* L. *Journal of Plant Science Research*, 3(1): 142.
- Zheng, W. and S. Y. Wang. 2001. Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agriculture Food Chemistry*, 49(11): 5165–5170.