EFFICACY OF DIETARY Moringa oleifera leaves supplementation on productivity, carcass traits, hematobiochemical parameters, antioxidants status and immune response in heat stressed growing rabbits.

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SUMMARY

This study was conducted to evaluate whether adding Moringa oleifera dry leaves powder (MODLP) as a natural anti-oxidants in the diet, has the potential to attenuate the negative effects of heat stress on growth performance, healthy status, anti-oxidant defense system and immunity of growing rabbits. Weaned NZW rabbits (n=120) were divided into four groups fed basal diet with 0, 100, 200 and 300 mg MODLP/kg. Growth performance parameters were recorded at age intervals from 5-13 wk. Caecal activity, carcass traits, hematological, serum biochemicals, antioxidant and immunity were determined at 13 wk of age. Results show that dietary addition of 200 mg MODLP/kg increased (P<0.05) pulse rate, growth performance parameters, net carcass weight, dressing rate and spleen percentages, beneficial bacteria (lactobacillus) count, hematological parameters, serum high-density lipoproteins, total antioxidant capacity, antibody titer and lysozyme. Abdominal fat percentage, E. coli count, and total cholesterol, triglycerides and low-density lipoproteins and thiobarbituric acid-reactive substances concentration reduced (P<0.05) 200 mg/kg group. The current study indicated that alleviation of adverse impacts due to heat stress on productivity, blood constituents, oxidative stress and immunity status, can be achieved in growing rabbits through dietary adding Moringa Oleifera dried leaves at a level of 200 mg/kg diet.

Keywords: Moringa oleifera, rabbits, heat Stress, productivity, antioxidative status, immunity.

INTRODUCTION

The productive performance of growing rabbits is markedly affected climatic conditions (El-Gindy et. al. 2017). Exposure of rabbits to high temperature (≥30ºC) induces severe heat stress (Okab et al. 2008), leasing to high cost for rabbit farmers, due decreasing to rabbit production of meat (Villalobos et al. 2008). Core body temperature, in term of rectal temperature (RT) is a good indicator for the animal response to fluctuations in air temperature (Marai et al. 2002). During exposure of Broiler chicks to heat stress, lipid oxidant increased following increasing the generation of free radicals, which elevates reactive oxygen species (ROS) formation and cellular oxidative stress induction (Hassan et al. 2016).

There are several natural plant anti-oxidants containing vitamin C, tocopherols, flavonoids and other phenolic compounds (Hassan et al. 2016). These compounds are important in elimination of heat stress impacts. Usage of these anti-oxidants in rabbit production are commercially accepted as strategy to improve the rabbit productive performance and health status (Ojo and Adetoyi, 2017). Moringa oleifera (MO) plant with their different parts in tropical and subtropical countries is with highly valued plant (Khalil and Korni, 2017). Phytochemical analyses of MO are rich in vitamins (C and B), carotene (α and β), minerals (Ca, K, Se, Zn, P and Fe), and essential amino acids (methionine and cysteine), polyphenols,
xanthins, and chlorophyll, and other phytochemicals with powerful anti-oxidant ability (Surai, 2002; Okwari et al., 2013). Therefore, MO leaves have ability to affect oxidative damage in animals by increasing anti-oxidant enzymes activity, decreasing lipid peroxidation, free radicals generation, and suppression of ROS formation (Osman et al., 2012), consequently improving meat quality (Giannenas et al., 2010). The MO leaves has properties as anti-tumor, anti-inflammatory, anti-oxidant, anti-ulcerous, anti-hyperlipidaemic and cholesterol lowering, anti-diabetic, anti-cancerous (Paul et al., 2018), anti-fungal and anti-bacterial (Bukar et al., 2010), which include 4-("L-rhamnopyranosyloxy) benzyl isothiocyanate, Niazimicin, Pterygospermin, Benzyl isothiocyanate, 4-("Lrhamnopyranosyloxy) and Benzyl glucosinolate (El-Kholy et al., 2018a).

Blood constituents reflected the animal physiology and health status under feeding trials and considered as an appropriate measures of the animal nutritional status (Terzungwe et al., 2013). Leaves of MO were found to effect on the haematological parameters and lipid profiles in animal blood (Serem et al., 2017). Additionally, rich nutrient profile of MO give this plant as a potential growth promoter and immunomodulatory effects (Paul et al., 2018). There are many attempts to use MO meal as a dietary inclusion in rabbit diets. However, usage of MO leaves as an anti-oxidant and anti-bacterial during growing stage in rabbits is rare. Therefore, this study aims to investigate the effects of Moringa oleifera leaves at levels of 0, 100, 200 and 300 mg/kg diet, as natural anti-oxidants and anti-biotics, on physiological response, growth performance parameters, caecal activity, liver and kidney functions, anti-oxidant status, immune response and carcass quality of NZW growing rabbits reared under heat stress condition.

MATERIALS AND METHODS

The present study was carried out at Experimental Rabbitary Farm, and Physiology and Biotechnology Laboratory, Animal Production Department, Faculty of Agriculture, Mansoura University, Egypt, during the period from August 2018 to October 2018. All procedures were approved by the Animal Care and Welfare Committee of the Institute.

Packaged Moringa oleifera dry leaves powder (MODLP) used for this study was purchased from the Moringa plantation unit of the Scientific Association of Moringa, National Research Center, Dokki, Egypt.

Environmental conditions

Indoor ambient temperature (AT) and relative humidity (RH %) were weekly recorded inside the rabbitry using electronic digital thermo-hygrometer. Average of maximum and minimum values of AT, RH and calculated thermal-humidity index (THI) during the experimental period are shown in Table 1. The THI was calculated according to the equation of Marai et al. (2001) as following:

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

Where db\(^\circ\)C = dry bulb temperature.

Values of THI <27.8 = absence of heat stress; 27.8 to 28.8 = moderate heat stress; 28.9 to 29.9 = severe heat stress, while >30.0 = very severe heat stress.

Table (1): Overall means of ambient temperature, relative humidity and thermal-humidity index (THI) during the experimental period.

| Item                      | Minimum     | Maximum     |
|---------------------------|-------------|-------------|
| Ambient temperature (°C)  | 25.17±0.98  | 33.61±1.00  |
| Relative humidity (%)     | 44.67±7.57  | 82.83±2.78  |
| THI value                 | 23.33±0.91  | 32.59±1.08  |

Experimental animals and management

Total of 120 male New Zealand white rabbits weaned at 5 weeks of age and averaged 688.5±2.135g initial body weight were disturbed according to body weight into four experimental groups (n=30 in each) were used in this study. Rabbits were individually housed in galvanized wire cages (35 × 35 × 60 cm) under similar hygienic conditions. Feed and clean water were offered ad libitum until 13 weeks of age.
Rabbits in the 1st group (G1) were fed a commercial pelleted diet without any supplementation (control diet), while 100, 200 and 300 mg of MODLP were added to the control diet of rabbits in the 2nd (G2), 3rd (G3) and 4th (G4) group, respectively. The control diet was formulated to cover all essential nutrient requirements of growing rabbits according to NRC (1977) as shown in Table 2. Chemical composition of the control diet and MODLP, on dry matter basis, according to the standard methods of AOAC (2012) is presented in Table 3.

Table (2): Ingredients of the control diet used for feeding rabbits in the experimental groups.

| Ingredient       | Ingredient (%) | Ingredient     | Ingredient (%) |
|------------------|----------------|----------------|----------------|
| Berseem hay      | 30.05          | Di-calcium phosphate | 1.60          |
| Barley grain     | 24.60          | Limestone      | 0.95           |
| Wheat brain      | 21.50          | DL-Methionine  | 0.20           |
| Soybean meal     | 17.50          | Sodium chloride| 0.30           |
| Molasses         | 3.00           | Premix         | 0.30           |

Each 1kg contains vitamin A (150,000 UI), vitamin E (100 mg), vitamin B1 (10 mg), vitamin K3 (21 mg), vitamin B2 (40 mg), vitamin B6 (15 mg), vitamin B12 (0.1 mg), pantothenic acid (100 mg), niacin (200 mg), biotin (0.5 mg), folic acid (10 mg), choline chloride (5000 mg), manganese (800 mg), zinc (600mg), iron (300 mg), copper (40 mg), iodine (500 mg), selenium (100 mg), and cobalt (100 mg).

Table (3): Chemical composition of the control diet and *Moringa oleifera* dry leaves.

| Chemical composition (%) | Control diet | *Moringa oleifera* dry leaves |
|--------------------------|-------------|------------------------------|
| Organic matter           | 91.42       | 89.55                        |
| Crude protein            | 17.36       | 19.70                        |
| Crude fiber              | 12.37       | 8.20                         |
| Ether extract            | 2.23        | 6.30                         |
| Nitrogen free extract    | 59.46       | 55.35                        |
| Ash                      | 8.58        | 10.45                        |

Analytical procedures for *Moringa oleifera* dry leaves

Anti-oxidant capacity of MODLP in terms of contents of vitamin A and C (Achikanu et al. 2013), alkaloids (Harborne, 1973), total phenolic (McDonald et al., 2001) and flavonoids (Kumaran and Karunakaran, 2007) in MODLP were determined. Also, activity of superoxide dismutase and catalase and glutathione concentration was determined using the method described by Malliga et al. (2014) as shown in Table 4. These analyzed for their active components of MODLP at Regional Centre for Food and Feed, RCFF, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

Table (4): Vitamin content, bioactive anti-oxidant compounds and phytochemical composition of *Moringa Oleifera* dry leaves.

| Item                                | Concentration |
|-------------------------------------|---------------|
| Vitamin content (mg/100 g):         |               |
| Vitamin E                           | 0.37          |
| Vitamin C                           | 0.25          |
| Anti-oxidant activity               |               |
| Glutathione (nmol/g)                | 25.80         |
| Superoxide dismutase (U/g)          | 12.20         |
| Catalase (U/g)                      | 95.00         |
| Phytochemical composition           |               |
| Total flavonoids (mg/g)             | 4.50          |
| Total polyphenols (%)               | 2.50          |
| Alkaloids (mg/100g)                 | 350           |
Experimental procedures

Physiological response

Rectal temperature (RT), respiration rate (RR) and pulse rate (PR) were recorded daily for 7 d in the morning (7 a.m.) before feeding during the last week of the experimental period (13 wk of age). The RT was measured to the nearest 0.1 °C by clinical thermometer into the rectum. While RR was measured by counting the flank movements for one min and PR was measured by femoral vein for one min.

Growth performance parameters

During the experimental period, live body weight (LBW) and daily feed intake (DFI) were weekly recorded, then average daily weight gain (ADG) and feed conversion ratio (FCR) were calculated at age intervals of 5-9, 9-13 and 5-13 wk. Also, dead rabbits were recorded, and then viability rate (VR) was computed and performance index (PI) was calculated during the entire length of the experimental period (5-13 wk). Performance index was calculated according to the following equation: \( PI = \frac{\text{Final LBW (kg)/FCR}}{100} \).

Blood sampling

Blood samples were collected at the end of the experimental period (13 wk of age), from five rabbits in each group during slaughter, into two test tubes for each animal, one with anticoagulant for haematological parameters and another test tube without anticoagulant for biochemical parameters in blood serum.

Haematological parameters, including hemoglobin (Hb) concentration, hematocrit value (Ht), count of red (RBCs), white (WBCs) blood cells and platelets were determined according to Provan et al. (2004).

The collected blood samples in tube without anticoagulant were centrifuged at 3500 rpm for 15 min to separate blood serum, which was stored at -20°C till assayed.

Concentration of total proteins (TP), albumin (AL), cholesterol, triglycerides, high density lipoproteins (HDL), low density lipoproteins (LDL), creatinine and urea as well as activity of aspartate (AST) and alanine (ALT) transaminases were determined in blood serum using commercial kits (Bio-Merieux, Laboratory Reagents and Products, France). Globulin concentration was obtained by difference between TP and AL concentration.

Anti-oxidants status, including total anti-oxidant capacity (TAC) and thiobarbituric acid-reactive substances (TBARS) were assayed in blood serum using commercially available kits (Bio Diagnostic Research).

Antibody titer

On day 15 after starting the experimental period, other five rabbits chosen from each group were immunized with 0.1 ml of a 2.5% Sheep Red Blood Cells (SRBCs) to measure antibody titer against SRBCs. Antiserum to SRBCs was collected 7 days post-challenge according to Wegmann and Smithies (1966). The agglutination titer was expressed as the \( \log_2 \) (Nelson et al., 1995). Also, Lysozyme activity was determined according to Schultz (1987).

Caecal activity

The caecal contents were immediately taken from the slaughtered rabbits from each group, and filtrated to estimate pH by digital pH meter, and then the caecal content was divided into two samples, one for estimation the total anaerobic bacteria count and Escherichia coli (E. coli) according to according to Collins et al. (1995), and lactobacilli bacteria count according to Kim and Goepfert (1971). Another sample was filtered through four folds of gauze for determination of total volatile fatty acids (TVFA) and ammonia nitrogen by steam distillation according to Warner (1964).

Carcass traits

The slaughtered rabbits (n=5) were fasted for 12 h and individually weighed to estimate pre-slaughter weight. Then, carcass traits were recorded. Carcass parts and body internal organs were weighed and calculated as percentage relative to LBW of each animal. Meat samples were taken from the trunk of each rabbit and minced, then dried at 60°C for 48 h and grounded for approximate chemical analysis according to AOAC (2012).
Statistical Analysis

All data was statistically analyzed by one-way ANOVA design using a software package (SAS, 2002). Completely randomized design was used based on the following model: \( Y_{ij} = \mu + G_i + e_{ij} \)

Where \( \mu \) = the overall mean, \( G_i \) = group (1…..4), and \( e_{ij} \) = residual error.

Viability rate and carcass traits percentages were statistically analyzed using Chi-Square test. The percentage values were transformed by arcsine values before analysis. The group significant differences were tested by Duncan’s multiple range test (Duncan, 1955) and set at \( P<0.05 \).

RESULTS AND DISCUSSION

Physiological response: The recorded values of THI during the experimental period (THI =32.59) indicated that rabbits in all groups were kept under very severe heat stress (THI value >30) according to Marai et al. (2001). Rabbits exposed to high ambient temperature (≥30 ºC) are severing from heat stress, because rabbits have difficulty to eliminate body heat under long period due to the unfunctional sweat glands (Marai et al., 2002).

During the experimental period, the rectal temperature (RT) and respiratory rate (RR) values were not affected significantly (\( P\geq0.05 \)) by MODLP treatments under heat stress condition. On the other hand, pulse rate (PR) was significantly (\( P<0.05 \)) higher in G3 than in G1 (control), but did not differ significantly from that in G2 (Table 5).

In agreement with the present results, El-Gindy et al. (2017) showed that RT and RR of growing rabbits were not significantly affected by feeding different levels of MO leaf meal under heat stress. In goats, the PR was higher (\( P<0.05 \)) in animals fed different levels of MO leaf meal than those fed the control diet (Babeker and Abdalbagi, 2015). It was reported that MO is of high and better nutritional value as signs of health and more productive animals (Addass et al., 2010). This increase may be attributed to increasing heat production as a results of more feed utilization in G3 than in other groups.

It is of interest to note that animals exposed to sever heat stress significantly increased thermoregulatory parameters, in terms rectum and skin temperatures (Marai et al., 2007). Generally, body temperature is affected by many factors like feed, environmental temperature, disease, sex and age (Guyton and Hall, 2000). The observed insignificant differences in RT and RR under heat stress may indicate no positive effect of MO addition on physiological response of rabbits in treatment groups.

| Item                        | Control (G1) | Moringa oleifera dry leaves powder level | SEM | P-value |
|-----------------------------|--------------|----------------------------------------|-----|---------|
|                             |              | G2 (100 mg/kg diet)                     |     |         |
| Rectal temperature (ºC)     | 39.73        | 38.33                                  | 1.027 | 0.8253  |
| Respiration rate (r/m)      | 47.35        | 47.82                                  | 0.512 | 0.8296  |
| Pulsed rate (pulse/min)     | 72.80        | 75.86ab                                 | 1.598 | 0.0453  |

\textsuperscript{a} and \textsuperscript{b}: Means in the same row with different superscripts are significantly different from each other (\( P<0.05 \)).

Growth performance: Addition of MODLP with different levels in the diets of all treatment groups (G2, G3 and G4) significantly (\( P<0.05 \)) increased LBW at 9 and 13 wk of age, reflecting significantly (\( P<0.05 \)) higher average daily gain (ADG) at 5-9, 9-13 and 5-13 wk of age intervals. Only at 9-13wk of age, daily feed intake (DFI) significantly (\( P<0.05 \)) increased by increasing level of MODLP up to 200 mg/kg diet. However, there were no significant differences in DFI at 5-9 and 5-13 of age intervals. All levels of MODLP significantly (\( P<0.05 \)) improved the FCR compared with the control group at all age intervals. This finding was associated with ADG and DFI in each group. Rabbits in G3 showed the highest ADG and DFI, while those in G2 and G4 showed higher ADG and lower DFI as compared to the control group. The recorded improve in LBW and FCR of treatment groups reflected significant (\( P<0.05 \)) increase in performance index (PI) in all treatment groups (G2, G3 and G4) as compared to the control group, being
These results indicated beneficial effects of dietary addition of MODLP (200 mg/kg diet) on growth performance of growing rabbits. These findings are in the same line with the previous published reports on growing rabbits, fed graded levels of MO leaf meal in the diet (Abubakar et al. 2015), treated with MO leaf as a natural anti-oxidant (El-Gindy et al., 2017) or aqueous MO leaves extract (El-Kholy et al., 2018a) and MO supplementation (Aljohani and Abduljawad, 2018), who found that MO could play a good impact on growth performance of rabbits.

### Table (6): Effect of *Moringa oleifera* dry leaves powder on growth performance of NZW growing rabbits at different age intervals.

| Item                              | Control (G1) | *Moringa oleifera* dry leaves powder level | SEM | P-value |
|-----------------------------------|--------------|-------------------------------------------|-----|---------|
|                                   |              | G2 (100 mg/kg diet) | G3 (200 mg/kg diet) | G4 (300 mg/kg diet) |     |       |
| Average live body weight (g)      | 688.93       | 684.67                     | 689.66                    | 690.87                    | 2.135 | 0.8212|
| At 5 wk (Initial)                 | 1372.78a     | 1417.58c                   | 1441.11b                  | 1436.52b                  | 1.554 | 0.001 |
| At 13 wk (Final)                  | 2076.96d     | 2153.92c                   | 2192.59a                  | 2179.32d                  | 1.807 | 0.001 |
| Average daily gain (g)            | 24.42d       | 26.17a                     | 26.82a                    | 26.59b                    | 0.029 | 0.001 |
|                                   | 25.15d       | 26.30a                     | 26.84a                    | 26.53b                    | 0.028 | 0.001 |
|                                   | 24.79d       | 26.23a                     | 26.83a                    | 26.56b                    | 0.021 | 0.001 |
| Average daily feed intake (g)     | 85.17        | 87.58                      | 87.67                     | 86.80                     | 0.916 | 0.210 |
| At 5–9 wk                         | 123.63c      | 126.75b                    | 128.85a                   | 125.64bc                  | 0.804 | 0.001 |
| At 5–13 wk                        | 95.29        | 96.17                      | 97.04                     | 95.15                     | 0.733 | 0.226 |
| Feed conversion ratio (g feed/g gain) |            |                            |                           |                           |       |       |
| At 5–9 wk                         | 3.49a        | 3.35b                      | 3.27b                     | 3.26b                     | 0.035 | 0.001 |
| At 9–13 wk                        | 3.78a        | 3.66b                      | 3.61b                     | 3.59b                     | 0.029 | 0.001 |
| At 5–13 wk                        | 3.84a        | 3.67b                      | 3.62b                     | 3.59b                     | 0.028 | 0.001 |
| Performance index (%)             | 54.17c       | 58.84b                     | 60.70a                    | 60.58b                    | 0.460 | 0.001 |
| Viability rate (%)                | 80           | 80                         | 90                        | 87                       | -     | -     |

\[a, b, c, and d : Means in the same row with different superscripts are significantly different from each other (P<0.05).\]

The yielded improvement of productive performance of growing rabbits may be attributed to that MO is rich in amino acids, vitamins and minerals (Faye et al., 2011) and the biological function of MO as a natural growth promoter (El-Badawi et al., 2014). Also, MO was used as anti-microbial agent (lipophilic compounds), which might improve nutrient utilization (Caceres et al., 1991). Moreover, phytochemical compounds (alkaloids, flavonoids and polyphenols) present in MO and anti-oxidant activity, may attach to the cytoplasmic membrane and remove free radicals, activate anti-oxidant enzymes and inhibit oxidases (Luqman et al., 2011).

**Caecal activity:** Results in Table 7 indicated significant (P<0.05) change of caecal activity in treated groups. Concentration of NH₃-N and total volatile fatty acids (TVFA) significantly (P<0.05) reduced only in G3 and G4 compared with G1. This trend of reduction reflected lower pH value, but the differences were not significant.

The observed decrease in NH₃-N and TVFA concentrations in G3 and G4 was in parallel with decreasing total count of bacteria causing by significant decrease in some harmful bacteria, particularly E. coli, but there was significant increase in beneficial bacteria count, such as lactobacillus. According to the previous findings, higher production of NH₃-N and TVFA occurred with the caecum with high rate of absorption via caecal wall, leading to marked reduction in their concentrations in the caecal contents.

These results indicated beneficial impacts of dietary addition of MODLP on the microbial ecology of the gastrointestinal tract of growing rabbits. These results are in agreement with the results of El-Kholy et al. (2018a) and Aljohani and Abduljawad (2018). The phytagenic compounds of MO have positive effects, such as the gut microflora regulation and the immune-response stimulation (Aljohani and
Abduljawad, 2018). The anti-microbial activity of MO may be attributed to the presence of bioactive compounds, inhibiting the microbial growth and interrupting some metabolic processes (Godstime et al., 2014). The present results indicated the anti-bacterial activity of MO on E. coli (Abalaka et al., 2012). Accordingly, MODLP could be a promising natural anti-microbial agent, beside the anti-oxidant activity (El-Kholy et al., 2018a).

Table (7): Effect of Moringa oleifera dry leaves powder on caecal activity of NZW growing rabbits.

| Item                        | Control (G1) | G2 (100 mg/kg diet) | G3 (200 mg/kg diet) | G4 (300 mg/kg diet) | SEM | P-value |
|-----------------------------|--------------|---------------------|---------------------|---------------------|-----|---------|
| NH₃-N (mg/dl)               | 30.40        | 27.20              | 23.40              | 24.80               | 1.255 | 0.010  |
| Total volatile fatty acids  | 6.71         | 6.77               | 6.81               | 6.85                | 0.023 | 0.004  |
| pH value                    | 5.94         | 5.93               | 5.89               | 5.92                | 0.015 | 0.173  |
| Total bacterial count (x10⁶) | 21.60        | 19.80              | 18.60              | 16.80               | 0.948 | 0.017  |
| Lactobacilli count (X10⁷)   | 6.25         | 7.48               | 8.96               | 7.05                | 0.164 | 0.001  |
| Escherichia coli count (X10⁶)| 6.66        | 5.71               | 3.81               | 4.92                | 0.092 | 0.001  |

a,b,c,d: Means in the same row with different superscripts are significantly different from each other (P<0.05).

Hematology, biochemicals and enzyme activity in blood serum: All hematological parameters (hemoglobin, RBCs, WBCs, platelets and hematocrit) significantly (P<0.05) improved by all levels of MODLP. Total cholesterol, triglycerides and low-density lipoproteins (LDL) concentrations in serum significantly (P<0.05) decreased in MODLP groups compared with control group, while HDL concentration was significantly (P<0.05) higher only in G3 than in other groups. On the other hand, the effect of MODLP treatment on serum total proteins (TP), albumin (AL), globulin (GL), creatinine and urea concentrations as well as aspartate (AST) and alanine (ALT) transaminases activities was not significant (Table 8).

The present results indicated no adverse effects of dietary adding MODLP on protein metabolites (TP, AL and GL) kidney (creatinine and urea) and liver (AST and ALT) functions. However, MODLP treatment had positive and pronounced effects on hematological parameters and lipid profile, particularly at level of 200 mg/kg diet.

The assessment of hematological parameters could be used to reveal the deleterious effect of some chemicals in plant extracts (Oyedemi et al., 2011). The present improvement in all hematological parameters in G3 indicated that MODLP had positive influence on blood hematological parameters in growing rabbits under Egyptian conditions (El-Kholy et al., 2018b). The observed increase in RBCs, platelets and hematocrit value may be attributed to that MODLP is rich in amino acids, vitamins, minerals, particularly, iron and contain strong antioxidants (Morsy et al., 2007; Faye et al., 2011).

In consistent with the obtained results in the current study, similar results were reported by several authors regarding the hematological parameters of growing rabbits (El-Gindy et al., 2017; Ojo and Adetoyi 2017; El-Kholy et al., 2018b; Aljohani and Abduljawawad 2018) or rats (Otitoju et al., 2014) treated with MO.

The marked reduction in lipid profile was reported by several authors on growing rabbits (Musa et al., 2014; Etchu et al., 2017; El-Kholy et al., 2018b). Concentration of LDL is a major component of the total cholesterol and considered as the main target of any lipid lowering agent (atherogenic lipoprotein) like MO leaves (El-Gindy et al., 2017). The MO contains bioactive components (β-sitosterol and phytosterol), which exhibit hypcholesterolemic effect (Mbhikay, 2012). Also, the hypolipidemia of MO may be due two mechanism actions by reducing cholesterol biosynthesis and absorption of dietary cholesterol (Hassarajani et al., 2007). On the other hand, the recorded insignificant effect of MO on protein metabolites is in good agreement with results reported on rabbits by Terzungwe et al. (2013). It is of interest to observe that enzyme activity in treatment groups is within a normal range, indicating intact muscle and organs like the liver and kidney of rabbits (Ewuola et al., 2015).

These results indicated that MODLP treatment had impact on hematology and lipid profile as well as normal protein metabolism and enzyme activity, reflecting good health status of growing rabbits.
Table (8): Effect of *Moringa oleifera* dry leaves powder on haematology, serum biochemicals and enzyme activity in NZW growing rabbits.

| Item                          | Control (G1) | *Moringa oleifera* dry leaves powder level | SEM | P-value |
|------------------------------|--------------|--------------------------------------------|-----|---------|
|                              | (100 mg/kg diet) | (200 mg/kg diet) | (300 mg/kg diet) |       |         |
| Hematological parameters:    |              |                                            |     |         |
| Hemoglobin (mg/dl)           | 9.53<sup>a</sup> | 9.79<sup>ab</sup> | 9.84<sup>a</sup> | 9.74<sup>b</sup> | 0.021 | 0.0001 |
| RBCs (x 10<sup>6</sup>/mm<sup>3</sup>) | 5.44<sup>d</sup> | 5.89<sup>c</sup> | 6.28<sup>d</sup> | 6.04<sup>b</sup> | 0.034 | 0.0001 |
| WBCs (x 10<sup>3</sup>/mm<sup>3</sup>) | 6.55<sup>a</sup> | 6.91<sup>b</sup> | 7.07<sup>c</sup> | 6.76<sup>a</sup> | 0.025 | 0.0001 |
| Platelets (x 10<sup>3</sup>/mm<sup>3</sup>) | 235.20<sup>c</sup> | 254.80<sup>a</sup> | 258.20<sup>a</sup> | 249.80<sup>b</sup> | 1.609 | 0.0001 |
| Hematocrit (%)               | 36.46<sup>c</sup> | 42.82<sup>a</sup> | 48.45<sup>a</sup> | 43.04<sup>b</sup> | 1.631 | 0.0010 |
| Serum biochemicals (mg/dl):  |              |                                            |     |         |
| Total proteins               | 6.19         | 6.16                                      | 6.134 | 6.15     | 0.022 | 0.3473 |
| Albumin                      | 3.99         | 3.95                                      | 3.92 | 3.94     | 0.029 | 0.4588 |
| Globulin                     | 2.20         | 2.21                                      | 2.21 | 2.22     | 0.035 | 0.9943 |
| Total cholesterol            | 128.20<sup>a</sup> | 118.60<sup>b</sup> | 110.60<sup>c</sup> | 120.20<sup>b</sup> | 1.643 | 0.0001 |
| Triglycerides                | 114.20<sup>a</sup> | 98.40<sup>b</sup> | 89.63<sup>c</sup> | 101.21<sup>b</sup> | 1.871 | 0.0001 |
| Low density lipoproteins     | 56.44<sup>a</sup> | 51.06<sup>b</sup> | 42.20<sup>c</sup> | 52.22<sup>a</sup> | 1.329 | 0.0001 |
| High density lipoproteins    | 58.66<sup>b</sup> | 65.20<sup>a</sup> | 70.60<sup>a</sup> | 64.60<sup>b</sup> | 2.724 |         |
| Creatinine                   | 1.50         | 1.46                                      | 1.43 | 1.44     | 0.097 | 0.9660 |
| Urea                         | 39.80        | 39.50                                      | 39.20 | 39.60     | 1.369 | 0.9915 |
| Enzyme activity (IU/l):      |              |                                            |     |         |
| Aspartate transaminases      | 33.80        | 33.64                                      | 33.24 | 33.42     | 1.094 | 0.9844 |
| Alanine transaminases        | 20.24        | 19.98                                      | 19.81 | 19.90     | 1.086 | 0.9928 |

<sup>a, b, c and d</sup>: Means in the same row with different superscripts are significantly different from each other (P<0.05).

**Antioxidant and immunity:** Treatment with MODLP at all levels significantly (P<0.05) increased total antioxidant capacity (TAC), while decreased thiobarbituric acid-reactive substances (TBARS) concentration in blood serum of growing rabbits, reflecting higher antioxidant defense system of treatment groups, in particular in G3. Also, MODLP treatment significantly (P<0.05) increased antibody titers against SRBCs and lysozyme concentration, representing higher immunity of treatment groups than the control one, being the highest (P<0.05) in G3 (Table 9).

Similarly, Ojo and Adetoyi (2017) and El-Kholy et al. (2018b) found that MO leaves treatment had beneficial effects on increasing TAC and reducing lipid peroxidation of growing rabbits. Also, in rats, Tuorkey (2016) showed that MO leaf extract administration decreased lipid peroxidation and increased TAC. These results may be attributable to the presence of flavonoids and polyphenols in MO leaves that can ameliorate oxidative stress (El-Kholy et al., 2018b). Also, MO leaves act as potentially antioxidant phytochemicals like caffeic and chlorogenic acids (Siddhuraju and Becker, 2003), which increase enzymatic antioxidants (Oseni and Idowu, 2014). Reducing TBARS concentration, as a product of lipid peroxidation, may be related to a decreased fat deposition by reducing the malate dehydrogenase and lipoprotein lipase activities, or increasing the hormone-sensitive lipase activity in the adipose tissue (Lu et al., 2007).

Generally, the MO leaves has anti-tumor and antiinflammatory properties (Paul et al., 2018). Concerning the observed improvement in immunity of rabbits treated with MODLP, similar results were reported on growing rabbits fed MO leaf meal under moderate heat stress (El-Gindy et al., 2017), broilers fed MO leaves (Olugbemi et al., 2010) and mice orally treated with MO leaves methanolic extract (Sudha et al., 2010).
Table (9): Effect *Moringa oleifera* dry leaves powder on antioxidant and immunity status of NZW growing rabbits.

| Item                   | Control (G1) | G2 (100 mg/kg diet) | G3 (200 mg/kg diet) | G4 (300 mg/kg diet) | SEM | P-value |
|------------------------|--------------|---------------------|---------------------|---------------------|-----|---------|
| Antioxidant status:    |              |                     |                     |                     |     |         |
| TAC (mmol/l)           | 1.97<sup>c</sup> | 2.18<sup>b</sup>   | 2.31<sup>a</sup>    | 2.12<sup>b</sup>    | 0.023 | 0.0001  |
| TBARS (nmol/ml)        | 1.23<sup>a</sup>     | 1.12<sup>b</sup>   | 1.01<sup>c</sup>    | 1.13<sup>b</sup>    | 0.024 | 0.0001  |
| Immunity status:       |              |                     |                     |                     |     |         |
| Antibody titer         | 5.31<sup>d</sup>    | 5.56<sup>c</sup>   | 6.02<sup>a</sup>    | 5.66<sup>b</sup>    | 0.031 | 0.0001  |
| Lysozyme (ug/mL)       | 103.80<sup>b</sup>  | 111.60<sup>ab</sup>| 118.40<sup>a</sup>  | 108.60<sup>b</sup>  | 2.820 | 0.0155  |

<sup>a, b, c and d</sup>: Means in the same row with different superscripts are significantly different from each other (P<0.05).

*Carcass traits*: Results showed that net carcass weight percentage and dressing rate in all treatment groups, and spleen weight percentage in G3 and G4 significantly (P<0.05) increased compared with control group. However, heart and tests weight percentages in G4, and abdominal fat weight percentage in G3, significantly (P<0.05) decreased as compared to other groups. Other carcass traits were not affected by MODLP treatment (Table 10).

Table (10): Effect of *Moringa oleifera* dry leaves powder on carcass traits of growing rabbits.

| Item                  | Control (G1) | G2 (100 mg/kg diet) | G3 (200 mg/kg diet) | G4 (300 mg/kg diet) | SEM | P-value |
|-----------------------|--------------|---------------------|---------------------|---------------------|-----|---------|
| Pre-slaughter weight (g) | 2093.00       | 2155.20             | 2193.00             | 2184.60             | -   | -       |
| Net carcass weight (%) | 51.26<sup>c</sup> | 53.36<sup>b</sup>   | 54.62<sup>a</sup>   | 53.55<sup>b</sup>   | 0.076 | 0.0001  |
| Head weight (%)        | 5.14          | 5.03                | 5.04                | 4.95                | 0.121 | 0.7529  |
| Liver weight (%)       | 2.59          | 2.54                | 2.53                | 2.50                | 0.100 | 0.9364  |
| Kidney weight (%)      | 0.58          | 0.57                | 0.58                | 0.56                | 0.075 | 0.9960  |
| Heart weight (%)       | 0.29<sup>a</sup> | 0.28<sup>b</sup>   | 0.28<sup>ab</sup>   | 0.27<sup>b</sup>    | 0.003 | 0.0010  |
| Spleen weight (%)      | 0.04<sup>b</sup> | 0.04<sup>b</sup>   | 0.05<sup>a</sup>    | 0.05<sup>a</sup>    | 0.001 | 0.0009  |
| Testes weight (%)      | 0.18<sup>a</sup> | 0.18<sup>a</sup>   | 0.18<sup>a</sup>    | 0.17<sup>b</sup>    | 0.001 | 0.0129  |
| Total edible offal’s (%) | 8.82          | 8.64                | 8.66                | 8.50                | 0.244 | 0.8453  |
| Dressing (%)<sup>*</sup> | 60.08<sup>a</sup> | 62.02<sup>a</sup>  | 63.30<sup>a</sup>   | 62.05<sup>b</sup>   | 0.280 | 0.0001  |
| Weight of in-edible offal’s (%) | 0.90        | 0.87                | 0.87                | 0.86                | 0.073 | 0.8934  |
| Digestive tract weight (%) | 0.181        | 0.178               | 0.177               | 0.175               | 0.002 | 0.2447  |
| Skin (%)               | 18.48         | 18.04               | 17.97               | 17.73               | 0.255 | 0.2557  |
| Blood (%)              | 2.87          | 2.79                | 2.77                | 2.76                | 0.171 | 0.9734  |
| Total in-edible offal’s (%) | 40.367      | 39.542              | 39.369              | 38.871              | 0.396 | 0.0990  |
| Abdominal fat (%)      | 0.86<sup>a</sup> | 0.83<sup>ab</sup>  | 0.78<sup>b</sup>    | 0.82<sup>ab</sup>   | 0.008 | 0.0001  |

<sup>a, b, c and d</sup>: Means in the same row with different superscripts are significantly different from each other (P<0.05).

<sup>*</sup> Based on weight of carcass and edible organs relative to pre-slaughter weight.
The important effect of pre-slaughter body weight on carcass characteristics. Therefore, as expected, carcass traits showed similar trend in treated groups compared with the control (Szendro et al., 1995). In accordance with the present results, Aljohani and Abduljawad (2018) showed that dietary MO supplementation improve carcass traits of growing rabbits. Also, El-Kholy et al. (2018a) found that Addition of aqueous extract of MO leaves in drinking water improved carcass characteristic of growing rabbits. Moreover, El-Badawi et al. (2014) concluded that inclusion of MODLP as a natural feed additive is highly recommended to enhance carcass dressing percentage of growing rabbits.

Such results might lead to support our suggestion that, addition of MODLP could boost feed utilization of rabbits by increasing the absorptive area of the small intestine in an action most likely to that of bacterial probiotics (El-Badawi et al., 2014). The observed decrease in abdominal fat percentages, especially in G3 is in association with reducing serum lipid profile as affected by MODLP treatment. In this respect, Toghyani et al. (2011) showed that MO supplementation was found to improve in beneficial bacteria (Lactobacillus) that reduce acetyl-CoA carboxylase activity, which is the rate-limiting enzyme in fatty acids synthesis.

Regard to the chemical composition the growing rabbit’s meat, MODLP had insignificant (P>0.05) effect on percentage of crude protein, ether extract, ash and water content (Table 11). Although the powerful effects of MO on improving the meat quality properties as mentioned by (Kahraman et al., 2015), the insignificant effect of MO on meat composition in our study was reported by Helal et al. (2017) on growing rabbits. Generally, MODLP had no adverse effects on the carcass traits or meat quality of growing rabbits.

### Table (11): Effect of Moringa oleifera dry leaves powder on meat composition of NZW growing rabbits.

| Item       | Control (G1) | Moringa oleifera dry leaves powder level | SEM  | P-value |
|------------|--------------|-----------------------------------------|------|---------|
|            |              | G2 (100 mg/kg diet)                     |      |         |
| Water content | 70.59       | 70.55                                   | 0.981| 0.9998  |
| Crude protein | 20.70       | 20.65                                   | 0.989| 0.9999  |
| Crude fat   | 7.100        | 7.18                                    | 0.079| 0.8696  |
| Ash         | 1.61         | 1.62                                    | 0.021| 0.3530  |
|            |              | G3 (200 mg/kg diet)                     |      |         |
| Water content | 70.50       | 70.50                                   | 0.9998|        |
| Crude protein | 20.72       | 20.66                                   |        |         |
| Crude fat   | 7.12         | 7.10                                    |       |         |
| Ash         | 1.66         | 1.63                                    |       |         |
|            |              | G4 (300 mg/kg diet)                     |      |         |
| Water content | 70.60       | 70.60                                   |       |         |
| Crude protein | 20.66       | 20.66                                   |       |         |
| Crude fat   | 7.10         | 7.10                                    |       |         |
| Ash         | 1.63         | 1.63                                    |       |         |

### CONCLUSION

Results of the current study suggest that dietary addition of Moringa oleifera dried leaves up to 200 mg/kg diet, as a natural antioxidant and antibacterial agent, could be better strategy to improve productive performance, bacterial loading, haematological and biochemical blood constituents, immune response and endogenous antioxidant status of growing rabbits under heat stress condition.

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Tأثير إضافة أوراق المورينجا أوليفيرا على الإجهاد الحراري على النمو، صفات الدم الهيماتو-بيوكيميائية، حالة مضادات الأكسدة والاستجابة المناعية في الأرانب النامية المجهدة حراراً

وائل أحمد خليل، رحاب فوزى صديق عبد الفتاح اسماعيل، ابراهيم طلعت الرطل

أجريت هذه الدراسة لتقييم ما إذا كان إضافة مسحوق أوراق المورينجا أوليفيرا الجافة كمضادات أكسدة طبيعية في العليقة، له القدرة على تخفيف الآثار السلبية الناتجة عن الإجهاد الحراري على أداء النمو، حالة الصحة، حالة مضادات الأكسدة والاستجابة المناعية للأرانب النامية. استخدمت في هذه الدراسة عدد 120 من الأرانب النامية (النيوزيلندي البيضاء)، قسمت إلى 4 مجموعات، حيثنُغيت الأرانب في المجموعة الأولى على العليقة كنترول (بدون إضافات)، بينما غذيت الأرانب في المجموعات الثانية والثالثة والرابعة على العليقة الكنترول مضادات الأكسدة 100، 200 و 300 مل جرام من مسحوق أوراق المورينجا أوليفيرا الجافة / كجم العليقة، على التوالي. تم تسجيل هجمة النمو على فترة العمر المختلفة من 5-13 أسبوع. تم تدريج نظام الإعرا، صفات الدم، خصائص الدم الهيماتولوجية والبيوكيميائية، حالة مضادات الأكسدة والمناعة في الدم في الأسبوع 13 من العمر. وقد أوضحت النتائج ان إضافة 200 مل جرام من مسحوق أوراق المورينجا أوليفيرا الجافة / كجم العليقة، كنترول، معدل التفسد، صفات الدم، خصائص الدم الهيماتولوجية، والبيوكيميائية، واستجابة المناعة (الثروتينات، والكولسترول، والكولسترول الثلاثي، والكولسترول الثلاثي، والكولسترول الثلاثي، والكولسترول الثلاثي)، وrack حمض الثيوبيلبيرون الفعال عند إضافة 200 مل جرام من مسحوق أوراق المورينجا أوليفيرا الجافة / كجم العليقة.

حصلت هذه الدراسة، أن التخفيف من الآثار الضارة الناجمة عن الإجهاد الحراري على الإنتاج، النمو من خلال إضافة 200 مل جرام من مسحوق أوراق المورينجا أوليفيرا الجافة / كجم العليقة.

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