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Haematological and Serum Biochemical Responses of Growing Rabbits to Aqueous Extract of *Moringa oleifera* Leaves in Drinking Water

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ABSTRACT

A total number of 64 mixed sex growing APRI rabbits aged 5 weeks and weighing 661.8±8.08 g was assigned randomly into four treatment groups to evaluate the impact of aqueous *Moringa oleifera* leaves extract (AMOLE) addition in drinking water. Treatments were 0 (control, G_1), 30 (G_2), 60 (G_3) and 90 (G_4) ml AMOLE/L. The study was lasted for 8 weeks during the growing period, from weaning age at 5 weeks to marketing age at 13 weeks. The results revealed that values of blood picture including hemoglobin, packed cell volume and neutrophils were significantly higher ($P \leq 0.01$) in growing APRI rabbits treated with AMOLE levels than those in the control group. The rabbits from the groups receiving AMOLE had lower total lipids, triglycerides and total cholesterol as compared with those from the control group. Also, results showed that there were no significant differences among the experimental groups in activity of serum transaminases (AST and ALT) and serum levels of creatinine and urea-N. The effect of AMOLE levels on total antioxidant capacity (TAC) was so clear, where treated groups showed significant ($P \leq 0.05$) increases in their TAC by about 17.5, 25.4 and 25.5%, respectively. The different administrated of AMOLE influenced significantly the lipid peroxidation (LPO) by decreasing serum mnalondialdehyde concentration. It can be concluded that addition of AMOLE to drinking water for growing rabbits has a beneficial effect on some aspects of their haematological and serum biochemical responses without any deleterious effects on liver and kidneys functions.

Keywords: Rabbits, Moringa leaves extract, blood, antioxidant

INTRODUCTION

The utilization of plants leaf extract in rabbit's production has found wide scientific and commercial acceptance as strategy to enhance the health status and performance of the animals (Djakalia *et al*., 2011; Ojo and Adetoyi, 2017). Extract of many plants with multi-phytochemicals have been documented to have antioxidant power with low monetary values (Zheng and Wang, 2001). Of these, *Moringa oleifera* which have different antioxidants with high levels such as phenolic acids (chlorogenic, ellagic, gallic and ferulic acid), glucosinolate and flavonoids like kaempferol, rutin and quercetin (Mbikay, 2012). Also, it has a valuable source of beta–carotene (vitamin A precursor) and the B-complex vitamins, C, D and K (Okwari *et al*., 2013). Moringa leaves are the most used part of *Moringa oleifera* plant which has been documented to have prebiotic and antioxidant phytochemicals, such as caffeic acid and chlorogenic acid (Siddhuraju and Becker, 2003).

The effect of leaves and seeds of *Moringa oleifera* plant were also examined by researchers for improving immune status and productive performance for growing rabbits (Ibrahim *et al*., 2014). Also, leaves of *Moringa oleifera* had been used as a natural antioxidant for its highly antioxidant substances such as polyphenols (Sreelatha and Padma, 2009); highly pepsin and total soluble protein which is acceptable to monogastric animals such as poultry (Kakengi *et al*., 2007), and has a useful effect on meat quality (Waskar *et al*., 2009), especially lipid peroxidation, one of the main reasons for the occurrence of meat quality deterioration, affecting negative impact on colour, texture, flavour and nutritional value (Giannenas *et al*., 2010).

Kachik *et al.* (2009) demonstrated that the negative effect of phytate and other anti-nutrients in several plants such as reduce the bioavailability of certain nutrients can be decreased by processing which can be done for maximum utilization of required nutrients. Makkar and Becker (1997) showed that a significant quantity of certain anti-nutrients, particularly saponins can be removal through aqueous and solvent extractions. Scientific information on this aqueous extract is limited. The purpose of this study was, therefore, to investigate the efficacy of aqueous *Moringa oleifera* leaf extract (AMOLE) as a natural source of antioxidants on haematological and serum biochemical parameters of growing rabbits.

MATERIALS AND METHODS

This study was conducted at the Rabbit Farm of Sakha Station, Animal Production Research Institute, Agricultural Research Center, Egypt.

Preparation of aqueous *M. oleifera* leaves extract (AMOLE)

Leaves of *M. oleifera* plant were collected and manually removed from the stem early in the morning at Dokki area of Giza Governorate. The *M. oleifera* leaves were cleaned and made free of sand and other impurities using distilled water. The leaves were air-dried in a laboratory for 5 days and ground into powder using an electric kitchen blender. Finely pulverized *M. oleifera* leaves weighing 300 g were poured into a 2.5 L flask and 1.5 L of distilled water were added. The resulting mixture was thoroughly homogenized and sieved with a cheesecloth and then filtered using Whatman filter paper (24 cm), and the extracts were placed in containers and diluted using distilled water (volume / volume) to form 30, 60 and 90 ml/1000 ml distilled water for treatments 2 to 4, respectively. The filtrate served to the experimental rabbits in their drinking water.

Experimental animals and management:

Sixty four Animal Production Research Institute (APRI) line rabbits (Egyptian line selected for litter weight at weaning according to Abou Khadiga *et al.*, 2010) were divided randomly into 4 experimental groups of 16 rabbits each (8 males + 8 females) of 5 wks of age with an average live body weight of 661.8±8.08 g. The four experimental groups were as follows: The control group (G_1) received basal diet and water without any supplementation, while groups 2, 3 and 4 received basal diet and water supplemented with 30 (G_2), 60 (G_3) and 90 (G_4) ml AMOLE /litter drinking water, respectively. Basal diet was formed to coverage all essential nutrient requirements for growing rabbits according to NRC (1977). Table 1 shows the formulation and nutrient composition of the basal diet.

Table 1. Composition and chemical analysis of basal diet

Ingredients	%	Calculated chemical analysis:[2]	%
Berseem hay	30.05	Dry matter (DM)	85.81
Barley grain	24.60	Crude protein (CP)	17.36
Wheat bran	21.50	Organic matter (OM)	91.42
Soybean meal (44% CP)	17.50	Crude fiber (CF)	12.37
Molasses	3.00	Ether extract (EE)	2.230
Limestone	0.95	Digestible energy (DE, kcal/kg)[(3)]	2412
Dicalcium phosphate	1.60	Calcium	1.243
Sodium chloride	0.30	Phosphorus	0.808
Mineral-vitamin premix[(1)]	0.30	Methionine	0.454
DL-Methionine	0.20	Lysine	0.862
Total	100		

[1]One kilogram of mineral–vitamin premix provided: Vitamin A, 150,000 UI; Vitamin E, 100 mg; Vitamin K_3, 21 mg; Vitamin B_1, 10 mg; Vitamin B_2, 40 mg; Vitamin B_6, 15 mg; Pantothenic acid, 100 mg; Vitamin B_{12}, 0.1 mg; Niacin, 200 mg; Folic acid, 10 mg; Biotin, 0.5 mg; Choline chloride, 5000 mg; Fe, 0.3 mg; Mn, 600 mg; Cu, 50 mg; Co, 2 mg; Se, 1 mg; and Zn, 450 mg.

[2]Calculated according to NRC (1977).

[3]Digestible energy (kcal / kg DM) = 4253 - 32.6 CF (% DM) – 114.4 Ash (% DM). According to Fekete and Gippert (1986).

Same managerial conditions were provided for all rabbits. Pelleted feed and fresh water were offered *ad libitum* throughout the experimental period (5 to 13 weeks of age).

Experimental procedure:

At the end of experimental period, four rabbits were randomly taken from each treatment, fasted for 12 hrs, weighed and slaughtered. Immediately after slaughtering, blood samples of growing rabbits were collected into clean centrifuge tubes; then sera were separated by centrifugation at 3000 r.p.m. for 20 minutes and kept in a deep freezer at -20 ^{0}C until later biochemical analysis. Heparinized blood samples were tested shortly after collection to determine blood pictures such as, red blood cells count (RBCs, 10^6/mm^3), white blood cells count (WBCs, 10^3/mm^3); different subclasses of WBC's (lymphocyte, neutrophils and monocytes percentages), hemoglobin (Hb, g/dl) concentration and packed cell volume (PCV, %) according to Provan *et al.* (2004). Total protein (TP, g/dl) and albumin (Alb, g/dl) levels in serum were determined using commercial kits (Bio-Diagonosis Co., Cairo, Egypt). Globulin (Glb, g/dl) concentration was estimated by subtracting the values of Alb from the corresponding values of TP. Serum creatinine (CR, g/dl), urea-Nitrogen (urea-N, g/dl) and total antioxidant capacity (TAC, mmol/l) were determined by the colorimetric method with commercial kits provided by Bio-Diagonosis Co (Egypt). Malondialdehyde (MDA, mmol/dl) as refer to lipid peroxidation was determined according to Buege and Aust (1978). Serum samples were analyzed for the activity of aspartate (AST, U/L) and alanine amino transferases (ALT, U/L) using commercial kits (Linear Chemicals, Barcelona, Spain) according to the manufacturer procedure. Also, the serum was assayed for total cholesterol, triglycerides and total lipids using standard protocol methods (Vogel, and Vogel, 1997).

Statistical analysis:

Data were statistically analyzed according to SAS (2000) computer program using the following fixed model:

$$Y_i = \mu + T_i + e_i$$

Where: Y_i = The observation; μ = Overall mean; T_i = Effect of treatments (i = 1, 2, 3 and 4); e_i= Random error component assumed to be normally distributed. Data in the form of percentages were converted to the corresponding arcsine values before being statistically analysed (Ewens and Grant, 2005). The Duncan's New Multiple Range Test was used to determine the differences among means. All data are presented as least square means.

RESULTS AND DISCUSSION

Hematological indices:

With regard to the haemogram illustrated in Table 2, values of haematological parameters including Hb, PVC and neutrophils were significantly higher ($P \leq 0.01$) in growing APRI rabbits treated with AMOLE levels than those in control group (G_1). However, these high values are remaining within normal limits. Some blood hematological values (RBC, eosinophils and basophil) in treated groups did not differ than that of the G_1 group (control). While, AMOLE administration significantly ($P \leq 0.05$) lowered the WBC values compared to control (G1) group. The trends resulting from AMOLE addition in the current study are in consistent with the results of other studies involving rabbits received 30, 60 and 90 ml AMOLE /kg BW (Ojo and Adetoyi, 2017). Also, Owolabi *et al.* (2012) and Otitoju *et al.* (2014) demonstrated that AMOLE improves the quantity of blood haemoglobin, when administered to rats.

According to Oyedemi *et al.* (2011), the assessment of blood picture could be used to reveal the deleterious effect of some chemicals in extracts of plant on the blood constituents of animals. In this study, it was observed that AMOLE had no significant influence on the RBC, eosinophils and basophil values. This shows that AMOLE can be suggested to use for growing rabbits under Egyptian conditions without any pathological deviation from the normal. However, the values of WBC obtained from treated rabbits showed as a slight depression which perhaps could be as a result of other factors related to experimental materials.

Table 2. Impact of different levels of AMOLE on blood hematological values of growing APRI-line rabbits.

Parameters	Control (G_1)	Moringa extract (ml/ litter water) 30 (G_2)	60 (G_3)	90 (G_4)	SEM	Sig.
Hemoglobin(g/ dl)	10.63^c	11.53^b	12.07^a	12.30^a	0.145	***
PCV (%)	38.10^c	40.27^b	41.27^b	43.33^a	0.537	***
RBCs($\times10^6$/ µl)	5.36	5.39	5.45	5.95	0.265	NS
WBCs($\times10^3$/ µl)	5.40^a	4.50^b	4.20^b	4.13^b	0.173	*
Lymphocytes(%)	47.33^a	43.33^{ab}	38.00^b	36.00^b	1.856	*
Neutrophils (%)	41.00^c	47.67^b	54.00^a	56.67^a	1.764	**
Monocytes (%)	6.67^a	4.67^b	4.33^b	4.00^b	0.577	*
Eosinophils (%)	4.00	3.33	3.00	2.67	0.333	NS
Basophil (%)	1.00	1.00	0.67	0.67	0.333	NS

SEM = Standard error of means, Sig.= significance

a, b, c Means in the same row with different superscript are significantly different (P≤0.05).

***** : Significant at 0.1% level of probability,**

****: Significant at 1% level of probability**

***: Significant at 5% level of probability, NS: Non-significant**

Metabolic profile :

Rabbits received AMOLE levels exhibited significant increases in TP, Alb and Glb as compared to control group (Table 3). These increases were pronounced with the high levels of AMOLE (G_3 and G_4) being, 9.4 and 10.2; 8.3 and 9.9; and 10.6 and 10.6%, respectively, but these increases are still within normal range. While, AMOLE administration in the G_2 did not significantly affect the TP, Alb and Glb values as compared to the control. The trends resulting from AMOLE addition in the current study are in agreement with those reported in a study involving broilers fed Moringa leaves meal or its extract (AbouSekken, 2015). In contrary to the present results, Ojo and Adetoyi (2017) reported that treated rabbits with AMOLE did not significantly influence serum levels of TP, Alb and Glb.

Concerning the effects of AMOLE levels on TP and its fractions (Alb and Glb), it can be explained the improvements in their profile in the present study especially with the high levels (G_3 and G_4). These improvements may be as a result to the resistance of animal to any physical or physiological stress. Furthermore, TP is a general indication of immune status (White *et al.*, 2002). Also, increased Glb concentration with AMOLE addition as observed in the current study may be an indicator to increase of immunity in rabbits since the liver will be able to synthesize enough Glb for immunologic action as mentioned by Sunmonu and Oloyede (2007). However, Glb level has been used as an indication of increased immunity and source of antibody production. El-Kholy *et al.* (2014) stated that high Glb level produces better resistant for diseases by improved immune status. This result is in harmony with those of Wallace *et al.* (2010) and Mbikay (2012), who found that several components in AMOLE are potent as immuno-stimulants.

The effect of AMOLE addition to the drinking water on lipid profiles of growing rabbits is shown in Table 3. The rabbits from the groups receiving AMOLE had lower total lipids, triglycerides and total cholesterol compared with those from the control group. This finding agrees with the results of a research conducted by Okwari *et al.* (2013). Also, results showed insignificant differences between G_3 and G_4 for values of total lipids, triglycerides and total cholesterol. In view of the fact that *M. oleifera* has been demonstrated to exhibit anti-cholesterolemic activity (Owolabi *et al.*, 2012; Okwari *et al.*, 2013) and its leaves have been reported to contain beta sitosterol, a phyto-constituent with potent cholesterol lowering ability (Owolabi *et al.*, 2012), so this may explain the significant decreased in lipid profiles for treated groups. Flavonoids and polyphenolic compounds found in AMOLE (Amaglo *et al.*, 2010; Okwari *et al.*, 2013) may be caused to impulse of immune function, reduced level of cholesterol; so may be play a role in the prevention of a number of chronic diseases such as cardiovascular disease and cancer in rabbits (Chang and Gershwin, 2000; Yousef, 2004).

Data in Table 3 shows the effect of AMOLE treatments on CR and urea-N levels as indicators of kidney function. The data indicated that there were no significant differences among the experimental groups in serum CR and urea-N. In both treated and untreated rabbits, levels of CR and urea-N concentrations are within the normal range of rabbits, implying that addition of AMOLE levels did not cause any damaging effects on kidney. Absence of significant difference in CR and urea-N in blood of control and AMOLE treated rabbits implies that despite receiving amounts of the AMOLE for a period of 56 days, AMOLE did not cause any major defect in renal function in treated rabbits. In this respect, Ouedraogo *et al.* (2013) stated that addition of 150-300 mg/kg of the aqueous-ethanol extract of Moringa leaves appears to be protective against gentamicin-induced nephrotoxicity, this may be due to detoxification effect of the extracts on kidney tissues.

Enzymatic profile:

The effect of AMOLE levels on liver enzymes (ALT and AST) is shown in Table 3. The results showed that the activity of serum transaminases (AST and ALT) in treated groups was not different from that of the control one. This result is in a good agreement with results found by AbouSekken (2015). El-Kholy *et al.* (2014) showed that increasing plasma total protein and its fractions (Alb and Glb), within the normal range, may reflect an improvement in the hepatic function. This phenomenon was observed in treated groups compared to control group. These results indicate normal liver function of rabbits received AMOLE. On the other hand, the antimicrobial properties of AMOLE, *i.e.* its ability to suppress the growth of most pathogenic bacteria, and this led to optimal enzyme activity. Similar to the other experiments, it was established that Moringa was not toxic even at higher doses (Ghebreselassie *et al.*, 2011). The AMOLE was also found to have a significant hepato-protective effect, which may be due to the presence of quercetin, a well-known flavonoid with a hepato-protective activity (Mishra *et al.*, 2011; Farooq *et al.*, 2012).

Table 3. Impact of different levels of AMOLE on some blood serum parameters of growing APRI-line rabbits.

Parameters	Control (G_1)	Moringa extract (ml/ litter water) 30 (G_2)	60 (G_3)	90 (G_4)	SEM	Sig.
Total protein (g/dl)	5.87^b	6.19^{ab}	6.42^a	6.47^a	0.135	*
Albumin (g/dl)	3.03^b	3.14^{ab}	3.28^a	3.33^a	0.064	*
Globulin (g/dl)	2.84^b	3.04^{ab}	3.14^a	3.14^a	0.079	*
Cholesterol (mg/dl)	74.37^a	71.95^b	69.88^c	69.55^c	0.651	**
Total lipids (g/dl)	313.0^a	299.3^b	287.7^{bc}	282.3^c	2.906	**
Triglyceride(mg/dl)	88.11^a	85.55^{ab}	83.73^b	82.45^b	1.044	*
Creatinine (mg/dl)	1.003	1.053	1.070	1.070	0.033	NS
Urea (mg/dl)	13.25	13.38	13.45	13.48	0.177	NS
Aspartate amino transferase (U/L)	22.24	22.05	21.82	21.91	0.475	NS
Alanine amino transferase (U/L)	15.59	1542	15.34	15.30	0.376	NS

SEM = Standard error of means, Sig.= significance

a, b, c Means in the same row with different superscript are significantly different (P≤0.05).

***** : Significant at 0.1% level of probability,**

****: Significant at 1% level of probability**

***: Significant at 5% level of probability, NS: Non-significant**

Lipid peroxidation and antioxidant defense system:

Data presented in the Table 4 revealed that different dietary levels of AMOLE influenced significantly the lipid peroxidation by decreasing MDA values. This result is in agreement with the findings of Ojo and Adetoyi (2017), who observed a lowered serum MDA level in rabbits given 30, 60 and 90 ml AMOLE /kg BW. Also, Luqman *et al.* (2012) showed that AMOLE decreased MDA, when administered to rats.

The low levels of lipid peroxidation in the treated groups (G_3 and G_4) as compared to those in G_1 coincided with the presence of low amount of total lipids in treated groups compared to G_1 as mentioned in Table 3. The AMOLE addition into drinking water for the growing rabbits decreased MDA concentration which may be related to a reduced the deposition of fat by decreasing the activities of malate dehydrogenase and lipoprotein lipase or increasing the hormone-sensitive lipase activity in the adipose tissue (Lu *et al.*, 2007). These results may be attributable to the presence of flavonoids that can ameliorate oxidative stress.

The effect of AMOLE levels on total antioxidant capacity (TAC) was very clear, treated groups with three levels (G_2, G_3 and G_4) showed significant (P≤0.05) increased in their TAC by about 17.5, 25.4 and 25.5%, respectively (Table 9). This result in harmony with the results obtained by Luqman *et al.* (2012) and Tuorkey (2016) who showed that TAC increased with an increase in AMOLE concentration. It can therefore be concluded that AMOLE addition at 60 or 90 ml/l can be used to impulse the antioxidant capacity of growing rabbits.

So, AMOLE may refer to as a noticeable source of compounds with health protective with antioxidant power. In this respect, Siddhuraju and Becker (2003) showed that Moringa leaves act as prebiotic effects and potentially antioxidant phyto-chemicals, such as caffeic acid and chlorogenic acid (Siddhuraju and Becker, 2003). Furthermore, many reports which showed that *M. oleifera* leaves are rich in flavonoids and polyphenols and have antioxidant activity (Atawodi *et al.,* 2010; Santos *et al.,* 2012).

Table 4. Impact of different levels of AMOLE on lipid peroxidation and total antioxidant capacity of growing APRI-line rabbits.

Parameters	Control (G_1)	Moringa extract (ml/ litter water) 30 (G_2)	60 (G_3)	90 (G_4)	SEM	Sig.
MDA (mmol/dl)$^{(1)}$	01.70^a	01.17^b	00.88^c	00.81^c	0.052	***
TAC (mmol/l)$^{(2)}$	31.33^b	36.80^a	39.30^a	39.33^a	0.498	***

SEM = Standard error of means, Sig.= significance

a, b, c Means in the same row with different superscript are significantly different (P≤0.05).

***** : Significant at 0.1% level of probability.**

(1) MDA: Malondialdehyde

(2) TAC: Total antioxidant capacity

CONCLUSION

Thus, it could be concluded that AMOLE could be successfully added to the drinking water of growing rabbits up to the level of 90 ml/l. In addition, 60 ml/l of AMOLE in growing rabbit's drinking water improved their haematological and serum biochemical parameters through enhancement of the antioxidant capacity of rabbits. Actually, further studies are needed to throw more light on the effect of this phytogenic additive on hormones status.

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خصائص الدم الهيماتولوجية والبيوكيميائية للأرانب النامية نتيجة لإضافة المستخلص المائي لأوراق المورينجا في ماء الشرب

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أجري هدا البحث باستخدام عدد 64 من أرانب الأبري النامية عمر 5 أسابيع وبمتوسط وزن 661.8 جرام تم تقسيمهم عشوائياً لأربع مجاميع معاملة لتقييم تأثير إضافة المستخلص المائي لأوراق نبات المورينجا لماء شرب الأرانب النامية على صورة وبعض الخصائص البيوكيمائية للدم. قسمت الأرانب إلى 4 مجموعات تجريبية متساوية، المجموعة الأولى بدون اضافة (مجموعة ضابطة)، بينما أضيف المستخلص المائي لأوراق نبات المورينجا لماء الشرب بنسب 30و 60 و90 مل/لتر ماء الشرب في المجاميع الثانية والثالثة والرابعة، على التوالي. استمرت الدارسة لمدة 8 أسابيع خلال فترة النمو "من عمر 5 أسابيع وحتى عمر التسويق وهو 13 أسبوع"أوضحت النتائج المتحصل عليها من هذه الدراسة أن قيم صورة الدم بما في ذلك الهيموجلوبين وحجم الخلايا المعبأة (PCV) وخلايا الدم البيضاء المتعادلة كانت أعلى معنوياً ($P \leq 0.01$) في أرانب الأبري المعاملة بمستويات من المستخلص المائي لأوراق نبات المورينجا مقارنة بتلك الموجودة في المجموعة الضابطة. وأظهرت الأرانب في المجموعات التي تتلقى المستخلص المائي لأوراق نبات المورينجا أقل في مستويات الدهون الكلية والدهون الثلاثية والكوليسترول الكلي مقارنة مع تلك التي في المجموعة الضابطة. كما بينت النتائج أنه ليس هناك فروق ذات دلالات إحصائية بين المجموعات التجريبية في نشاط الإنزيات الدالة على وظائف الكبد ومستويات مصل الكرياتينين واليوريا-N. كان تأثير المستخلص المائي لأوراق نبات المورينجا على القدرة الكلية المضادة للأكسدة واضح جداً حيث أظهرت المجموعات المعاملة زيادة معنوية ($P \leq 0.05$) في القدرة الكلية المضادة للأكسدة بنسب 17.5 و 25.4 و 25.5٪ على التوالي. كما كان لإضافة مستويات مختلفة من المستخلص المائي لأوراق نبات المورينجا أثراً ملحوظاً في أكسدة الدهون (الليبيد بيروكسيد) عن طريق خفض تركيز مستوى المنالوندیالدیهید (MDA). ويمكن استنتاج أن إضافة المستخلص المائي لأوراق نبات المورينجا إلى مياه الشرب للأرانب النامية له تأثير مفيد على بعض جوانب استجاباتها الدموية والكيميائية الحيوية من دون أي آثار ضارة على وظائف الكبد والكلى.