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Research Article

Effect of Aqueous Extract of *Moringa oleifera* Leaves on Some Production Performance and Microbial Ecology of the Gastrointestinal Tract in Growing Rabbits

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Abstract

Objective: This study was conducted to determine the effect of aqueous *Moringa oleifera* leaf extracts (AMOLE) on growth performance and carcass characteristics of rabbits. **Methodology:** 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 effect of aqueous *Moringa oleifera* leaves extract (AMOLE) added in water. 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/L drinking water, respectively. The study was lasted for 8 weeks during the growing period, from weaning age (at 5 weeks) to marketing age (at 13 weeks). **Results:** The results revealed that *Moringa* leaves extract at its highest levels significantly ($p\leq 0.05$) increased the final body weight, daily weight gain and improved feed conversion. The effect of *Moringa* leaves extract on carcass traits was so clear, where supplemented groups showed significant ($p\leq 0.05$) increased the carcass percentage. Gastrointestinal tract and abdominal fat percentages were decreased ($p<0.01$ and $p<0.05$, respectively) by supplementing AMOLE in drinking water. Also, results showed that pathogenic bacteria (*Escherichia coli* and *Clostridium* sp.) decreased ($p<0.001$) by supplementing AMOLE in drinking water. The highest and pronounced increase of net revenue (16.8%) was observed for rabbits received 90 mL AMOLE/L (G_4). **Conclusion:** Addition of AMOLE in drinking water for growing rabbits, enhanced the growth performance and improved the microbial ecology of the gastrointestinal tract with high profitability. From economic point of view 90 mL L^{-1} *Moringa* extract is recommended for growing APRI rabbits, which showed the best results.

Key words: Rabbits, *Moringa* leaves extract, productive performance, microbial ecology

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The plants and leaf extracts are widely utilized in animal feed to improve the health status and growth performance of animals[1,2]. Leaf extracts also have appetizing and digestion-stimulating properties and antimicrobial effect[3].

Several plant extracts have different phytochemicals and diverse antioxidant activity with low economic importance such as *Moringa oleifera*[4].

Moringa oleifera contains the certain phytochemicals, which have been reported to possess anti-bacterial features and these include: (1) 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate, (2) Niazimicin, (3) Pterygospermin, (4) Benzyl isothiocyanate, (5) 4-(α-Lrhamnopyranosyloxy) and (6) Benzyl glucosinolate. Phytochemical analyses of *Moringa oleifera* have shown that its leaves are particularly rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, as well as antioxidants such as β-carotene, vitamin C and flavonoids[5,6]. In addition, the phytochemical screening of aqueous extract of *M. olifera* revealed the presence of alkaloids, flavonoids, gallic tannins, phenols, saponins and catecholic compounds and steroids indicating the presence of pharmacologically important phytochemicals[7].

Moringa oleifera leaves are reported to have potential prebiotic effects and potentially antioxidant phytochemicals, such as chlorogenic acid and caffeic acid[8]. In addition, Kakengi *et al*.[9] showed that *Moringa* leaves rich in pepsin and total soluble protein which is suitable to monogastric animals such as poultry. Kachik *et al*.[10] reported that the presence of phytate and other anti-nutrients can reduce the bioavailability of certain nutrients. Makkar and Becker[11] reported that significant quantity of anti-nutritional factors, particularly saponins, can be removed through solvent and aqueous extractions. Information on this extract is limited.

The objective of this study was, therefore, to determine the effect of aqueous *Moringa oleifera* leaf extracts (AMOLE) on growth performance and carcass characteristics of APRI line growing rabbits.

MATERIALS AND METHODS

This study was carried out at the Rabbits Farm of Sakha Station, Animal Production Research Institute, Agriculture Research Center, Egypt.

Preparation of aqueous *M. oleifera* leaves extract (AMOLE): Fresh leaves of *M. oleifera* were collected early in the morning at Dokki area of Giza Governorate. The *M. oleifera* leaves were manually removed from the stem, cleaned and made free of sand and other impurities using distilled water. The fresh leaves were blended into powdered using an electric kitchen blender. Finely pulverized *M. oleifera* leaves weighing 300 g was poured into a 2.5 L macerating flask and 1.5 L of distilled water was added. The resulting mixture was thoroughly homogenized and sieved with a cheese cloth and then filtered using Whatman filter paper (24 cm). Resulting filtrate was stored in the freezer (4 or -20°C) until further analysis.

Experimental animals and management: Sixty four APRI line rabbits (Egyptian line selected for litter weight at weaning according to Khadiga *et al*.[12] were divided and assigned randomly into four experimental groups of 16 rabbits each (8 males+8 females) of 5 weeks 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/L drinking water, respectively. Basal diet was formulated to cover all essential nutrient requirements for growing rabbits according to NRC[13]. Table 1 shows the formulation and nutrient composition of the basal diet.

All rabbits were kept under the same managerial conditions. Feed and water were offered *ad libitum* throughout the experimental period (5-13 weeks of age).

Experimental procedure: Live body weight (BW, g), daily feed intake (DFI, g) and number of dead rabbits were recorded weekly. Daily weight gain (DWG, g), feed conversion rate (FCR, g g^{-1}) and mortality rate (MR, %) were calculated weekly. Economic efficiency (EE, %) was calculated according to price marketing during 2016. Also, relative growth rate (RGR, %) and performance index (PI, %) were calculated on a group basis[14]:

$$\text{Relative growth rate (\%)} = \frac{(W2\text{-}W1)}{[1/2\ (W2+W1)]} \times 100$$

Whereas:

W1= Initial body weight (g)
W2 = Final body weight (g)

$$\text{Performance index (\%)} = \frac{\text{Final live body weight (kg)}}{\text{Feed conversion ratio}} \times 100$$

Table 1: Composition and chemical analysis of basal diet

Ingredients	%	Calculated chemical analysis**	%
Berseem hay	30.05	Dry matter (DM)	85.810
Barley grain	24.60	Crude protein (CP)	17.360
Wheat bran	21.50	Organic matter (OM)	91.420
Soybean meal (44% CP)	17.50	Crude fiber (CF)	12.370
Molasses	3.00	Ether extract (EE)	2.230
Lime stone	0.95	Digestible energy (DE, kcal kg^{-1})***	2412.00
Di-calcium phosphate	1.60	Calcium	1.243
Sodium chloride	0.30	Phosphorus	0.808
Mineral-vitamin premix*	0.30	Methionine	0.454
DL-methionine	0.20	Lysine	0.862
Total	100.00		

*One kilogram of mineral-vitamin premix provided: Vitamin A: 150,000 UI, Vitamin E: 100 mg, Vitamin K3: 21mg, Vitamin B1: 10 mg, Vitamin B2: 40 mg, Vitamin B6: 15 mg, Pantothenic acid: 100 mg, Vitamin B12: 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. **Calculated according to NRC (1977). ***Digestible energy (kcal kg^{-1} DM)= 4253-32.6 CF (% DM)-114.4 Ash (% DM). According to Fekete and Gippert[15]

At the end of growing period (13 weeks of age), four rabbits were taken randomly from each treatment, fasted for 12 h, weighed and slaughtered to estimate some of carcass traits according to the method described by Blasco *et al.*[16]. Carcass parts were presented as a percent of live body weight. Samples of cecum content were taken individually from rabbits of each group and filtrated to estimate pH and cecum microflora. Total anaerobic bacteria count and *Escherichia coli* (*E. coli*) were estimated according to the method described by Collins *et al.*[17] and lactobacilli bacteria count according to Kim and Goepfert[18]. In addition, cecum pH was measured by using pH meter in filtrate cecum content. Ammonia nitrogen concentration was determined as described by Conway[19].

Statistical analysis: Data were statistically analyzed according to SAS[20] computer program using the following fixed model:

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

Where:

Y_i = 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 presented as percentages were transformed to the corresponding arcsine values[21] before being statistically analyzed. The differences among means were tested using Duncan's new multiple range test[22]. All data are presented as least square means.

RESULTS AND DISCUSSION

Productive performance: Effect of different levels of AMOLE on final body weight (BW), DWG, DFI, FCR, RGR (%) and PI (%) of APRI line rabbits are presented in Table 2. Values of final BW, DWG and FI at 5-13 weeks of age were higher significantly ($p \leq 0.01$) for rabbits in G_2, G_3 and G_4 than those in control group. The proportional increments were 3.5, 7.7 and 8.3% for final BW; 5.6, 11.7 and 12.4% for DWG and 3.4, 8.0 and 8.2% for FI, for the three levels, respectively. These results are in agreement with El-Gindy *et al.*[23]. While, Alabi *et al.*[24] showed negative effect of AMOLE for final BW and DWG in broilers. Also, present study showed that values of final BW and DWG in G_3 group (60 mL AMOLE/L) was not different from G_4 group (90 mL AMOLE/L).

Results show that DFI significantly increased as AMOLE levels increased. The improvement in FCR at 5-10 weeks was significantly high among the three levels of AMOLE; the best FCR was recorded in G_3 (3.6%) and G_4 (3.8%) compared to control (G_1) which means better returns on investment. These findings were confirmed by Alabi *et al.*[24], who found that addition of 90 mL and 120 mL of AMOLE/L in broilers diet produced better FCR than control. It has also been documented that high packed cell volume (PCV) and high hemoglobin content (Hb) "as mentioned in Table 5" are associated with high feed conversion ratio[25].

Results indicate that addition of AMOLE caused significant differences ($p \leq 0.05$) in the values of RGR and PI. The highest values of RGR and PI (%) were observed in G_3 and G_4, without any significant differences between G_1 and G_2.

The antimicrobial (lipophilic compounds) and antioxidant (polyphenols, tannins, anthocyanin, glycosides compound) present in AMOLE may attach to the cytoplasmic membrane and remove free radicals, activate antioxidant enzymes and inhibit oxidases thus, making this elements more available for the poultry to use[26]. Furthermore, the synergy between individual bioactive compounds in AMOLE may be an important feature of their action which may affect broad aspects of physiology, such as nutrient absorption[27,28].

Table 2: Effect of *Moringa* leaves extract water supplementation on growth performance of growing APRI-line rabbits from 5-13 weeks of age

	Moringa extract (mL L^{-1} water)					
Parameters	Control (G_1)	30 (G_2)	60 (G_3)	90 (G_4)	SEM	Sig.
Number of animals	16.00	16.00	16.00	16.00	-	-
Initial body weight (g)	661.90	662.20	661.60	661.30	8.084	NS
Final body weight (g)	2103.00^{c}	2176.00^{b}	2265.00^{a}	2277.50^{a}	10.40	***
Daily weight gain (g)						
5-9 weeks	24.77^{c}	26.61^{b}	27.63^{ab}	28.28^{a}	0.386	***
9-13 weeks	26.57^{b}	27.60^{b}	29.71^{a}	29.44^{a}	0.369	***
5-13 weeks	25.67^{c}	27.10^{b}	28.67^{a}	28.86^{a}	0.163	***
Feed intake (g/day)						
5-9 weeks	63.16^{c}	65.13^{b}	67.73^{a}	68.34^{a}	0.362	***
9-13 weeks	101.40^{c}	105.00^{b}	109.30^{a}	109.70^{a}	0.377	***
5-13 weeks	82.28^{c}	85.05^{b}	88.53^{a}	89.04^{a}	0.265	***
Feed conversion ratio						
5-9 weeks	2.569	2.455	2.468	2.422	0.037	NS
9-13 weeks	3.821	3.808	3.694	3.736	0.046	NS
5-13 weeks	3.207^{a}	3.140^{ab}	3.091^{b}	3.086^{b}	0.029	*
Relative growth rate	81.80^{b}	83.86^{ab}	86.66^{a}	86.71^{a}	1.108	**
Performance index (%)	49.57^{b}	51.28^{b}	54.00^{a}	54.26^{a}	0.721	***
Mortality (%)$^{\#}$	6.25	6.25	6.25	-	-	-

SEM: Standard error of means, Sig.: Significance, ***Significant at 0.1% level of probability, **Significant at 1% level of probability, *Significant at 5% level of probability, NS: Non-significant. a, b, c, Means in the same row the different superscript are significantly different (p<0.05). #Chi-square test

Table 3: Effect of *Moringa* leaves extract water supplementation on carcass traits of growing APRI-line rabbits

	Moringa extract (mL L^{-1} water)					
Parameters (%)	Control (G_1)	30 (G_2)	60 (G_3)	90 (G_4)	SEM	Sig.
Carcass	45.80^{b}	47.70^{a}	48.90^{a}	49.10^{a}	0.348	**
Liver	4.78^{a}	4.23^{ab}	3.74^{b}	3.95^{ab}	0.328	*
Kidney	0.66	0.69	0.69	0.73	0.042	NS
Heart	0.43^{a}	0.40^{ab}	0.37^{ab}	0.35^{b}	0.018	*
Total edible parts (TEP)	51.60^{b}	53.00^{a}	53.70^{a}	54.10^{a}	0.254	**
Abdominal fat	0.98^{a}	0.78^{b}	0.53^{c}	0.56^{c}	0.046	**
GIT	19.90^{a}	18.30^{ab}	17.30^{b}	17.10^{b}	0.549	*

SEM: Standard error of means, Sig: Significance, A, B, C, Means in the same row the 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, GIT: Gastrointestinal tract

Some carcass characteristics: Table 3 shows that dietary AMOLE levels increased the values (p<0.05) of carcass and total edible parts percentages as compared to control group. However, liver, heart, abdominal fat and GIT percentages of the rabbit received AMOLE significantly decreased compared with that of the control group (G_1). Also, the results showed that the effect of dietary treatments on kidney was not significant. These results are in agreement with Nuhu[29].

The increase in carcass for treated groups may be related to the increase in growth performance. Therefore, pre-slaughter weight is considered to be one of the most important factor affecting carcass traits in rabbits. Szendro *et al.*[30] reported the important effect of pre-slaughter body weight on carcass traits. It seems that the literature is still sparse on the effect of AMOLE on rabbit's carcass traits.

It is a common practice in feeding trials to use weights of some internal organs like the kidneys as indicators of toxicity if there was any serious effect of anti-nutritional factors on them being major detoxification organs[31]. It was obvious in this study that the weight of organs such as kidney was insignificantly affected by AMOLE treatments as a result of absent of anti-nutritional factors. On the other hand, significant decreased of liver and heart weights as increased of AMOLE concentration need histological study to discuss this point.

Gastrointestinal tract and abdominal fat percentages were decreased (p<0.01 and p<0.05, respectively) by the addition of AMOLE. The lower gastrointestinal tract percentage could be explained by the increase in the carcass percentage. This was also observed by Amber *et al.*[32], who reported the lowest abdominal fat percentage value for poultry treated with prebiotic. No clear mechanisms have been reported responsible for the reduction of lipid synthesis by prebiotics and herb oligosaccharides. It might be due to increase in beneficial bacteria such as *Lactobacillus* that decrease the activity of acetyl-CoA carboxylase, which is the rate-limiting enzyme in fatty acids synthesis[33].

Table 4: Effect of *Moringa* leaves extract water supplementation on caecum content and microbial activity of growing ARPI-line rabbits

Parameters	Control (G_1)	*Moringa* extract (mL L^{-1} water) 30 (G_2)	60 (G_3)	90 (G_4)	SEM	Sig.
NH_3 (mmol L^{-1})	10.90^a	9.05^b	8.28^b	8.01^b	0.330	**
Total bacterial count ($\times 10^6$)[1]	10.83^c	12.63^{bc}	14.07^{ab}	15.27^a	0.467	**
Lactobacilli ($\times 10^5$)[1]	5.27^c	7.63^b	9.03^a	9.27^a	0.318	***
Escherichia coli ($\times 10^4$)[1]	5.63^a	3.97^b	2.84^c	2.29^c	0.219	***
Clostridium spp.[1]	6.33^a	4.53^b	2.27^c	2.37^c	0.425	***

SEM: Standard error of means, Sig: Significance, ***Significant at 0.1% level of probability, **Significant at 1% level of probability, a, b,⋯.e, Means in the same row with different superscripts are significantly different (p<0.05). Germ counts expressed in CFU g^{-1} caecal digesta

Table 5: Effect of *Moringa* leaves extract water supplementation on economical traits of APRI LINE rabbits at 13 weeks of age

Parameters	Control (G_1)	*Moringa* extract (mL L^{-1} water) 30 (G_2)	60 (G_3)	90 (G_4)
Average feed intake (kg/head)	4.608	4.763	4.958	4.986
Price kg^{-1} diet (L.E.)	3.966	3.966	3.966	3.966
Total feed cost (L.E.)	18.274	18.889	19.662	19.774
Average weight gain (kg/head)	1.438	1.518	1.606	1.616
Selling price (L.E.)*	35.942	37.942	40.142	40.406
Net revenue (L.E.)[#]	17.668	19.053	20.480	20.632
Relative revenue (%)	100.00	107.800	115.900	116.800

Other conditions like management are fixed. Ingredients price (L.E. per ton) at 2016 were: 4100 yellow corn, 4000 barley, 2000 berseem hay, 3600 wheat bran, 7000 soybean meal (44%), 250 limestone, 12000 premix, 60000 methionine, 40000 lysine, 1000 di-calcium phosphate, 3500 molasses, 250 salt. Adding 100 L.E./ton for pelliting. *Price of kg live body weight was 25 L.E., [#]Net revenue = Selling price-total feed cost

Caecum content and microbial activity: Results indicate that dietary AMOLE levels had some effects on the microbial ecology of the gastrointestinal tract in growing rabbits. Table 4 shows that supplementing AMOLE in drinking water of rabbits significantly affected caecum content. Ammonia content was significantly decreased (p<0.001) by supplementing AMOLE in drinking water. Total count of bacteria increased in caecum content for rabbits in control group (G_1) as compared to those received AMOLE in drinking water. Pathogenic bacteria (*Escherichia coli* and *Clostridium* sp.) decreased (p<0.001) by supplementing AMOLE in drinking water. Similarly, Mateos *et al.*[34] and Amber *et al.*[32] indicated that supplementation of rabbit feeds with certain prebiotics increased volatile fatty acids in the caecam and decreased the caecal ammonia concentration. In addition, to stimulate the beneficial microflora of the gut, prebiotics may prevent the adhesion of mucosa pathogens and stimulate the immune response[35]. These results are in agreement with the results of Amber *et al.*[32], who found that addition of some prebiotics in rabbit diets reduced number of total bacterial count (especially pathogenic bacteria) in caecum content of rabbits. The most susceptible organisms to the antibacterial activity of *M. oleifera* was *E. coli*[36]. Therefore, *M. oleifera* could be a promising natural antimicrobial agent.

On the other hand, the antioxidant properties of AMOLE may affect the alimentary canal through antimicrobial activity.

The antimicrobial activity of *M. oleifera* leaf may be due to the presence of an array of phytochemicals[36]. Bukar *et al.*[37] identified, most importantly, the presence of a short polypeptide named 4 (ά-L-rhamnosyloxy) benzyl-isothiocyanate in *M. oleifera* which may act directly on microorganisms and result in growth inhibition by disrupting cell membrane synthesis or synthesis of essential enzymes.

Economical evaluation: Data concerning economical evaluation indicate an increase of net and relative revenue for rabbits treated with AMOLE levels compared to those untreated (Table 5). The highest increase (16.8%) in net revenue was observed for rabbits of G_4 (90 mL AMOLE/L). This result is in harmony with those of Abou Sekken[38] who used AMOLE in the drinking water of broilers. Also, results showed that total feed cost increased by the addition of AMOLE, as a result of increased in feed intake. Also, selling price was increased by the addition of AMOLE in drinking water. This increase in selling price in treated groups may not only due to increase in average weight gain (kg/head), but also reduction in mortality rate in the same groups.

CONCLUSION

It is concluded that AMOLE can successfully be incorporated into the drinking water of growing rabbits up to the level of 90 mL L^{-1}. However, the addition of 60 mL of AMOLE/L in drinking water, improved the production performance and microbial ecology of the gastrointestinal tract with high profitability of growing rabbits, under Egyptian environmental conditions.

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