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IMPACT OF DIETARY SUPPLEMENTATION with MORINGA (*Moringa oleifera*) ON PRODUCTIVE PERFORMANCE, PHYSIOLOGICAL RESPONSE AND IMMUNITY OF GROWING RABBITS

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Forty five growing New Zealand White (NZW) rabbits aged five weeks and weighed in average 784.7±16.65 gm were randomly distributed by weight into five treatment groups (9 rabbits each), the experiment lasted 35 days. The 1st group fed a basal diet free from Moringa Olifera Leaf Meal (MOLM) and served as control. The 2nd, 3rd, 4th and 5th groups were fed the same basal diet supplemented with dried MOLM at levels of 0.1, 0.2, 0.3 and 0.4%, respectively.

The results showed that: *final live body weight and daily body weight gain were lower (P≤0.05) in rabbits fed different levels of MOLM compared with the control. Feed conversion ratio (FCR) was the best (P≤0.05) with rabbits fed 0.3% MOLM compared with the control and other groups. The FCR recorded 2.95; 3.17; 2.98; 2.83 and 3.24 for rabbits fed diet supplemented with 0.0; 0.1; 0.2; 0.3 and 0.4% MOLM, respectively.*

The mortality rate (%) recorded zero% in all experimental groups, meaning that supplementation of different levels of MOLM up to 0.4% of the formulated diet had no adverse effects on the rabbits health.

The levels of blood serum total protein, albumin and globulin were within the normal physiological range in all rabbit groups, whereas lysozyme levels were significantly (P≤0.01) higher in rabbit groups fed diets supplemented with moringa and positively correlated with the level of moringa leaves in the diet. Moringa oleifera leaves meal could be added up to 0.4% in the diet of growing rabbits to improve their immunity response and health status.

Another experiment with using large number of rabbits and higher levels of MOLM should be carried out to ascertain the actual effects of feeding moringa leaves on immunity response and health status of rabbits.

Key words: *Moringa oleifera* leaves meal, rabbits, growth, immunity, blood serum proteins, lysozyme.

Moringa Oleifera is a *perennial* plant, commonly known as "The Miracle tree or Horseradish tree" and is considered the most widely distributed species of *Moringaceae* family. Moringa plant has many of medicinal, industerial and agricultural uses. The leaves are highly nutritional and contain significant quantities of vitamins (A, B complex and C), minerals (calcium, potassium, phosphorus and iron) and high quality protein (Chen *et al.*, 2009). Moringa leaves are free from the toxic metals such as mercury, arsenic and cadmiumand antinutritive substances ,making the possibility of incorporation it into the poultry and animal diets without adverse effects on production and health status (Frederick, 2010 and Dougnon *et al*, 2012). *Moringa leaves* contain high levels of vitamin C and E, carotenoids, flavonoids and selenium, make it potential as antioxidant (Moyo *et al.*, 2012).

Inclusion of MOLM in the diet of quail have been improved the quality of quail meat products in the pre-slaughter or post-slaughter stages (Valeria and Williams, 2011). The author interpreted this improvement to the antioxidants compounds present in MOLM. Moringa leaves contain antibacterial and antifungal compounds, thus reduce the activity of pathogenic bacteria and moulds and improves the digestibility and feed utilization of ingested feeds by chickens (Gaia, 2005).

Moringa leaves possess antitumor, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic and hepatoprotective activities (Ritu *et al.*, 2011).

Moreover, it was reported that *Moringa oleifera* leaves effectively prevent the morphological changes and oxidative damage in human and animals by enhancing the activities of antioxidant enzymes, reducing the intensity of lipid peroxidation and inhibiting generation of free radicals (Guevara *et al.*, 1999). Moringa leaves are also used to promote the immune system against infections (Anwar *et al.*, 2007) and its extracts have positive effects on hematological parameters of rabbits (Caceres *et al.*, 1992).

This study aimed to verify the nutritional impact of feeding different levels of *MOLM* in the diet on some productive and physiological performance traits and immunity response in rabbits.

MATERIALS AND METHODS

Moringa leaves were obtained from Egyptian Scientific Association of Moringa, National Research Center. The fresh leaves were harvested, air-dried under shadow until the moisture of collected leaves reached 10%. The dry

leaves were finally milled, sieved (1 mm mesh) and stored in a well tight polyethylene bags at room temperature 25°C.

Forty five unsexed growing New Zealand White (NZW) rabbits aged five weeks old with an average body weight of 784.7±16.65 gm were randomly distributed by weight into five experimental groups (9 rabbits in each) and lasted 35 days. The rabbits were housed individually in galvanized metal wire cages equipped with feeding and water troughs, where the first group of rabbits was given a basal pelleted diet free from MOLM and served as control.

The basal diet (Table 1) was formulated to contain: 16.72% crude protein, 2.95% crude fat, 13.07% crude fiber and 2490 kcal/kg feed as DE. The 2^{nd}, 3^{rd}, 4^{th} and 5^{th} groups were given the same basal diet supplemented with MOLM at levels of 0.1, 0.2, 0.3 and 0.4%, respectively.

Initial and final live body weight (IBW and FBW), daily feed intake (DFI), daily body weight gain (DBWG), daily feed conversion ratio (FCR) and mortality rate % were recorded. At 70 days of age, blood samples were taken randomly into dried and cleaned tubes from three rabbits in each group from the ear vein. Blood samples were centrifuged at 3000 r.p.m. for 20 min to separate blood serum. Blood serum samples were subjected to biochemical analysis for determination of total protein (Sonnerwirth and Jarrett, 1980), albumin (Doumas *et al.,* 1971), and globulin was calculated by difference.

Zinc sulphate turbidity test was used to gain a rough estimation of the amount of immunoglobulins present in the serum. A small amount of serum was added to a zinc sulfate solution and allowed to incubate at room temperature for 1 h. Zinc sulfate will cause precipitation of the immunoglobulins, which makes the solution cloudy instead of clear. A lack of cloudiness indicates a lack of immunoglobulins (Johnson *et al.,* 1995). The turbidity is expressed as ZST units, which in turn indicate the amount of immunoglobulin present in the sample. Blood lysozymes level, a parameter of non-specific immunity was determined according to the established procedure of Amadori *et al.,* (1997).

Statistical analysis:

Data were analyzed using the General Linear Model (GLM) procedure of SAS (2004) as following model:

$$Y_{ijk}=\mu + T_i + e_{ijk},$$

Where: Y_{ijk} = An observation; μ = Overall mean; T_i = Moringa level; and e_{ijk} = Experimental error.

The significant differences among means were tested by using Duncan's Multiple Rang Test (Duncan, 1955).

Table 1. The ingredients and chemical composition of the pelleted diet fed to rabbits during the experimental period

Ingredients	**%**
Clover hay	30.00
Wheat bran	26.38
Barley grain	23.00
Soybean meal (44% CP)	16.00
Molasses	3.00
Lime stone	1.00
Sodium chloride	0.50
Vitamins & Mineral Premix *	0.30
Moringa ***	----
Total	**100.00**
Calculated chemical composition **	
Crude protein (CP)%	16.72
Ether extract (EE)%	2.95
Crude fiber (CF)%	13.07
Digestible energy (Kcal/Kg diet)	2490.00

*** Each one kg of Premix contains**: 150000 IU of Vit. A, ,100 mg Vit. E, 21 mg Vit. K3, 10 mg Vit.B_1, 40 mg Vit. B2, 15 mg Vit. B_6, 0.1 mg Vit. B_{12}, 200 mg Niacin, 100 mg Pantothenic acid, 0.5 mg Biotin, 10 mg Folic acid, 5000 mg Choline chloride, 450 mg Zn, 600 mg Mn, 0.3 mg Fe, 50 mg Cu, 0.25g I, 1 mg Se.

** Digestible energy calculated according to NRC (1977).

*** *Moringa oleifera* leaves meal were added to the basal diet at levels of 0.1, 0.2, 0.3 and 0.4%, respectively.

RESULTS

Productive performance:

Results of final live body weight (FBW), daily body weight gain (DBWG), daily feed intake (DFI) and daily feed conversion ratio (FCR) for the experimental period are presented in Table 2.

Final body weights and average daily weight gain were significantly ($P \leq 0.05$) lower in rabbit groups fed different levels of MOLM compared with those of the control. The FBW recorded 2033.70, 1826.24, 1896.16, 1925.91 and 1929.79 gm for rabbits fed diet supplemented with 0.0, 0.1, 0.2, 0.3 and 0.4% MOLM, respectively.

Daily feed intake had no obvious trend for growing rabbits fed diet supplemented with different levels of MOLM, however it was the highest for rabbits fed 0.4% MOLM and the lowest with those fed 0.3% MOLM compared with the other groups.

Average FCR at the end of the experiment were 2.95, 3.17, 2.98, 2.83 and 3.24 for those fed diet supplemented with 0.0, 0.1, 0.2, 0.3 and 0.4% MOLM, respectively, indicating that feed conversion ratio was improved with rabbits fed diet supplemented with 0.3% moringa compared to the other groups. Mortality rate recorded zero for all groups through the experimental period.

Blood analysis:

Table 3 and Figure 2 show that blood serum total protein levels for growing rabbits were not affected significantly by feeding the different levels of MOLM. Total serum protein values were 5.66, 5.94, 5.49, 6.73 and 6.39 g/dl for those fed 0.0, 0.1, 0.2, 0.3 and 0.4% MOLM, respectively.

Albumin levels were increased as MOLM level increased, except for those fed 0.2% MOLM diet. The values of albumin were 1.57, 1.79, 1.60, 2.78 and 2.22 for rabbits fed diet supplemented with 0.0, 0.1, 0.2, 0.3 and 0.4% moringa, respectively (Table 2 and Figure 1).

No obvious trend was detected for the effect of treatments on blood serum globulin levels. However, feeding 0.3% MOLM diet significantly ($P \leq 0.05$) elevated the level of globulin in the blood serum compared to the control and the other treatment groups (Table 2 and Figure 1).

Lysozymes levels in growing rabbits were significantly ($P \leq 0.05$) increased by increasing the level of MOLM supplementation in the diet. The lysozymes levels were 6.33, 61.20, 259.66, 300.00 and 213.50 µg/ml for rabbits fed 0.0, 0.1, 0.2, 0.3 and 0.4% MOLM, respectively.

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DISCUSSION

Results are in agreement with the findings of Yassmine *et al.,* (2017) who found that significant decrease in daily feed intake with an increase of MOLM up to 0.2%. They also, showed that inclusion of MOLM improves the FCR compared with control group, but this improvement didn't significant. The daily feed intake and the FCR values were similarly improved with increasing level of MOLM, but the differences were not statistically significant (Frederick, 2010). The FCR ratio obtained in this study were nearly of those (2.63-4.00) reported by earlier researchers in the tropics (Ayers *et al*., 1996; Okorie, 2003); but were lower than that of 5.32 – 5.63 reported by Eustace

Figure 1: Serum proteins of growing rabbits fed different levels of Moringa

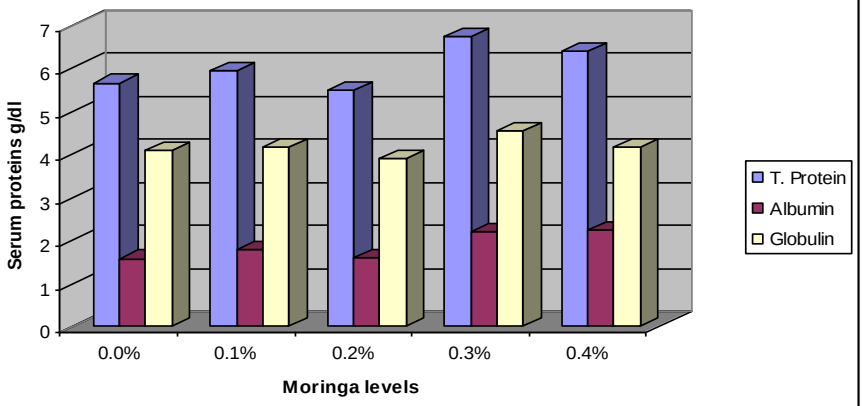
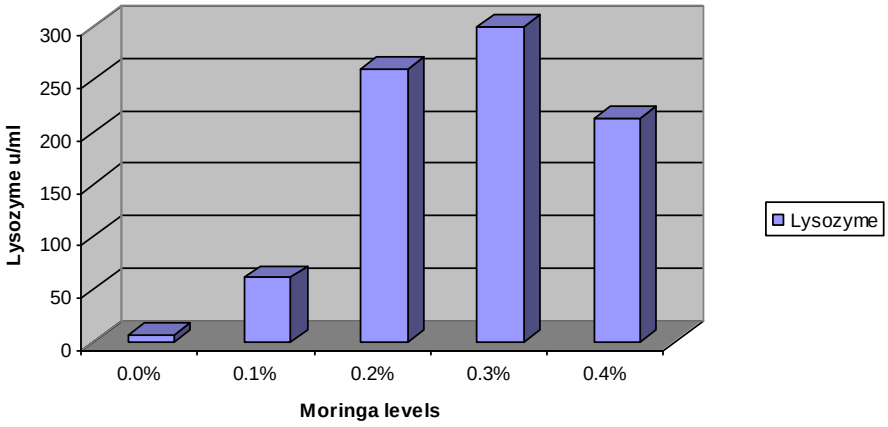


Figure 2: Serum lysozyme of growing rabbits fed different levels of Moringa



et al. (2003). The generally poor FCR ratio obtained were probably due to the relatively low growth rates.

On the other hand, results are in disagreement with the findings of El-Badawi *et al*., (2014) who found that supplementation of *Moringa olifera* dry leaves to growing rabbits diets at 0.15 or 0.30% improved protein utilization efficiency and Bouatene *et al*., (2011) who found that average body weight, body weight gain, growth rate and feed efficiency were the best ($P \leq 0.05$) in rabbits supplemented in the diet with MOLM at levels of 1.5 or 3% compared with control group. Also, Dougnon *et al.,* (2012) who found that the best weight gain and feed conversion ratio were obtained in rabbits fed pellets of MOLM as a substitute for 10 and 15% of pelleted commercial ration. Feed conversion ratio recorded 4.24 and 4.02 for rabbits fed 10 and 15% MOLM, respectively. The rabbit's carcass yield ranged from 60.6 to 64.5%. Pellets of *M. Oleifera* can substitute the commercial feed at a level of 15% with a positive effect on weight gains and carcass characteristics of rabbits. Yassmine *et al.,* (2017) found that rabbit's final body weight was increased significantly by MOLM supplementation in the diet at 0.2 and 0.3% compared with control group.

Concerning blood analysis, results are in agreement with those reported by Frederick (2010) who found that feeding rabbit's levels of 5, 10, 15 or 20% moringa leaves meal (MOLM) to substitute soybean meal in diet did not alter the blood hematological and biochemical characteristics such as total protein, albumin and globulin. Yassmine *et al.,* (2017) found that hemoglobin was not significantly affected by different levels of MOLM.

Concerning immunity, Olugbemi *et al.,* (2010) reported that *Moringa oleifera* leaves had a beneficial effect on the immune responses and improve intestinal health of broilers. Moringa oleifera leaves are widely used for its antimicrobial activity (Suarez *et al.,* 2005) and its pharmacological properties (Mehta *et al.,* 2003). It also contain sufficient quantities of carotene, ascorbic acid, iron, methionine and cystine (Makkar and Becker, 1996). Yassmine *et al.,* (2017) found significant increase in WBCs values in the rabbit groups fed diets containing MOLM compared with the control group. High counts of WBCs enhance adaptability to local environment and disease prevalent condition (Soetan *et al.,* 2013). Yassmine *et al.,* (2017) showed that MOLM was immunomodulatory, it stimulated numerically IgG and significantly IgM immune response of growing rabbits. Sudha *et al.,* (2010) showed that methanol extract of moringa leaves given orally to mice at doses of 250 and 750 mg/kg stimulated both cellular and humoral immune responses.

Moscati *et al.,* (2008) reported that lysozyme at level of 27.19 µg / ml is a strong antibacterial enzyme (against Gram positive) and has a synergic

action with immune humeral response, and factors of the serum complement. Moscati, *et al.*, (2008) recorded that lysozyme value of 27.19 μg/ml as an innate immunity indicator was higher than those reported by Carroll and Martinez, (1979) in healthy rabbits (0.85 μg/ml). Moscati *et al*., (2005) reported values of rabbit serum lysozyme from 4.8 to 11.0 μg / ml. Innate immunity plays an important role in preventing infection as a first-line defense and also contributes antigen presenting cells that activate the adaptive immune response, which is specific and powerful (Tizard, 2013).

The positive relationship between lysozymes levels and MOLM supplemented diets confirming the potential effect of moringa on improving the immune response and diseases resistance in rabbits. This is might be due to the presence of considerable levels of antioxidants (Vit. E and C, carotenoids and selenium) in MOLM which enhance the release of lysozymes (Klasing and Leshchincky 2000 and Gallois *et al.*, 2005). Recently, Dabbou *et al.*, (2016) found that lysozyme and the complement system are interesting indicators of how innate immunity functions. They reported that their work has been performed in a standard environment without infection, stress or other factors that could influence immune responses. Therefore, it is difficult that experiments conducted in normal conditions could result in a significant effect on immunity, despite supplementation with an immunomodulating agent. In this study it could be concluded that supplementation of *Moringa oleifera* Leaf Meal MOLM in the growing rabbits diet had a significant effect on rabbit physiological responses and immunity. Humeral and innate immunity act by releasing high amount of lysozyme and increase of albumin ratio.

Conclusively, from these results it could be concluded that moringa supplementation might play a role as immunity enhancer and diseases resistance for growing rabbits. Moringa oleifera leaves meal could be added up to 0.4% in the diet of growing rabbits to improve their immunity response and health status.

Another experiment with using large number of rabbits and higher levels of MOLM should be carried out to ascertain the actual effects of feeding moringa leaves on growth performance, immunity response and health status of rabbits.

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تأثير إضافة مسحوق أوراق المورينجا (مورينجا أوليفيرا) الجافة في العليقة على الأداء الإنتاجى والإستجابة الفسيولوجية ومناعة الأرانب النامية

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إستخدم فى هذه التجربة عدد 45 أرنب نيوزيلندي ابيض عمر خمسة أسابيع ومتوسط وزن 784.7 ± 16.65 جم تم توزيعها عشوائيا حسب الوزن إلى خمس مجموعات تجريبية متقاربة (9 أرانب في كل مجموعة)، حيث تغذ ت المجموعة الأولى على عليقة أساسية خالية من مسحوق أوراق المورينجا، في حين تم تغذي ة المجموعات 2، 3، 4، 5 على نفس العليقة الأساسية مضافا إليها مسحوق أوراق المورينجا بنسبة 0.1، 0.2، 0.3، 0.4٪ على التوالى.

وقد أظهرت النتائج ما يلى:

1- أظهرت تغذية الأرانب النامية على علائق مضافا إليها مسحوق أوراق المورينجا أنها لا تؤدى لزيادة فى وزن الجسم النهائي ومعدل الزيادة اليومية في وزن الجسم حيث تفوقت مجموعة المقارنة على المجاميع المعاملة.

2- إنخفض معدل إستهلاك الغذاء اليومى وتحسنت كفاءة التحويل الغذائي فى المجموعة التجريبية المضاف لها مسحوق أوراق المورينجا مقارنة بمجموعة الكنترول وكانت الفروق معنوية على مستوى معنوية 0.05

3- لم يكن هناك أي إختلافات معنوية عند مستوى معنوية (0.05) بين مجموعة المقارنة و المجموعات المضاف لها مسحوق أوراق المورينجا في بروتينات الدم الكلية، بينما زادت معدلات كل من الألبيومين والجلوبيولين فى الدم نتيجة التغذية على

مسحوق أوراق المورينجا مما يعكس تحسن الحالة الفسيولوجية والمناعية للأرانب المعاملة بالمورينجا.

4- إرتفع الليسوزيم بشكل كبير جدا فى سيرم دم الأرانب المعاملة بمستويات مختلفه من المورينجا مقارنة بالكنترول.

التوصية: إستخدام مسحوق أوراق المورينجا كاإضافة غذائية بمستوي حتي 4 % ربما يلعب دورا مهما فى تحسين مناعة الأرانب النامية ، ومن الأهمية بمكان إقامة تجربة أخري باستخدام عدد أكبر من الأرانب في كل مجموعة ومستويات أعلي من أوراق المورينجا المجففة لكي يمكن الحكم الصحيح علي تأثير التغذية علي كفاءة أداء الأرانب والإستجابة المناعية والحالة الصحية للأرانب.