**MENOUFIA JOURNAL OF ANIMAL, POULTRY AND FISH PRODUCTION**

https://mjapfp.journals.ekb.eg/

# EFFECT OF MORINGA OLIFERA SUPPLEMENTATION ON IMMUNE RESPONSE, OXIDATIVE STRESS IN BARKI RAMS

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Received: Jun. 11, 2022 Accepted: Jun. 25, 2022

**ABSTRACT:** Fifteen Barki ram were used to study the effect of MOL or MOSC supplementation in the ration on IR, MAD, TAC, IgG, IgM, WBCs, serum transaminases and renal function. The rams were divided into 3 groups. Rams in G1 were fed a basal diet supplemented with MOL (0.5% of CFM/day), rams in G2 were fed a basal diet supplemented with MOSC (10% of CFM/day) while G3 were fed a basal diet without supplementation. The results indicated that supplementation with MOSC increased antibodies after immunization, TAC and decreased MAD in blood samples of rams fed a ration containing MOSC or MOL compared to those rams fed moringa-free rations in contrast to the activity of MAD. The highest IgG recorded in blood samples of rams fed MOl followed by that measured in rams fed MOSC, the lowest was for rams fed a Moringa-free ration. However, the highest IgM recorded in rams fed MOSC followed by that in rams fed a Moringa-free ration and the lowest for rams fed ration with MOL. MOSC or MOL supplementation slightly increased WBC and neutrophil cells, supplementation of rams with MOSC significantly reduced creatinine and GPT compared to those supplemented with MOL or the control group. It can be concluded that feeding supplementation ration of Barki rams with MOSC can increase antibody production, WBCs, neutrophils, TAC and decreased MAD, having a positive effect on kidney function and liver enzymes, and enhances the immunity status of rams.

**Key words:** Moringa Olifera, supplementation, Immune response, Barki rams.

## INTRODUCTION

Nowadays, many different natural medicinal plants are used all over the world as nutritional supplements in animal and poultry foods to improve their yield and performance as an alternative to antibiotics and other synthetic medicines (Bedi et al., 2016., Mahanta et al., 2017., Wang et al., (2018). It should be noted that the addition of synthetic drugs to animal foods led to the appearance of drug residues in the food products of these animals, which affected the people who consumed these products and increased health risks (Hao et al., 2014). In addition, an increase in antibiotic resistance was recorded in animals and humans who have used these synthetic drugs (Gholamiandehkordi et al., 2009; Bannam et al., 2011 and Zidaric et al., 2012).

Moringa oleifera (MO) is one of thirteen known species belonging to the family Moringaceae that grows in many countries (Olson., 2002., Cuellar-Nuñez et al., 2018). MO is considered a natural food has many medicinal benefits and used as a good source of high-quality feed for farm animals (Fahey, 2005 & Singh et al., 2018). At the same time, Mahfuz & Piao (2019) conclude that MO has therapeutic properties for a variety of infections, and positively regulates the immune system because it contains several proteins and peptides, vitamins, and minerals as well as antioxidants that help the immune system protect healthy cells, in addition to containing factors Anti-diabetic and anti-tumor. Furthermore, Farouk et al. (2007) and Pakade et al. (2013) reported that Moringa contains more than 40 natural antioxidant compounds that have the ability to scavenge free radicals.

Several studies have shown that different parts of this plant have beneficial properties and are recognized as nutritional and medicinal value (Dhakad et al., 2019, Liang et al., 2019),. In this

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regard, many chemical compounds have been isolated from Moringa leaves such as flavonoids, phenolic acid, glucosinolates, isothiocyanates, as well as Moringa leaves possess various biological activities, including hypocholesterolemic, antidiabetic, blood pressure lowering, and hypolipidemic effect, while terpenes have only been isolated from Moringa pods (Palada & Chang, 2003, Anwar et al., 2007., Bichi, 2013., Baldisserotto et al., 2018 and Dhakad et al., 2019). In addition, Jaiswal et al., (2009) and Mahfuz & Piao., (2019) concluded that the aqueous extract of M. oleifera supplementation showed a strong immune modulating effect, can modulate B-cell activation and stimulate IgM, IgA and IgG production (Ojeka et al. 2016)., in addition, it leads to a significant increase in the level of white blood cells and neutrophils (Gupta et al., 2010). *The current study aimed to evaluate the effect of Moringa supplementation (leaves or seeds) in rations of Barki rams on: immune response (IR),* changes in antioxidant indices such as Malondialdehyde (MAD) and total antioxidant capacity (TAC), *immunoglobulins (IgG, IgM) as well as white blood cell count and leukocyte differentiation. Serum transaminases activity (GOT, GPT), renal function (creatinin and urea) were also evaluated.*

## MATERIALS AND METHODS

Fifteen adult Barki rams aged 17-20 months, weighing 56.5 ± 0.57 kg live body weigh were randomly selected from the sheep experimental flock of the Animal Production Department, Faculty of Agriculture, Minoufia University, Shebin El-Kom, Egypt to carry out this study. These rams were divided into three comparable groups (5 rams each), and were housed in three separate groups of closed, well-ventilated and lighted pens. The animals were healthy, free of internal and external parasites; the experiment was conducted from December 2020 to May 2021.

Animals were fed in groups, and provided with a basic diet including concentrate plus wheat straw plus berseem (Trifolium alexandrium), the feed allowance was according to the NRC (1985). Rams in group 1 (G1) were fed a basal diet supplemented by moringa olifera leaves (MOL) at a concentration of 0.5 % of concentrate feed mixture /day, whereas rams in groups 2 (G2) fed a basal diet supplemented by moringa olifera seed cake (MOSC) at a concentration of 10% whereas group 3 (G3) were fed a basal diet with no supplements. Rations were offered to all animals twice a day of 08:00 a.m. and 3.00 p.m., the feeding allowance were adjust monthly according to changes in body weight. Fresh water was available ad lib.

### *Moringa by-products preparation:-*

Moringa oleifera (MOL) leaves were processed as described by (Salem et al., 2020) as follows: Moringa oleifera (MOL) leaves were collected from the horticultural farm of the Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt. Moringa leaves with their stems and twigs were gathered, the leaves separated from the stems using a manual method, and the moisture content of the leaves was determined using the standard method of oven drying. Samples were heated to 60-70 C until the moisture content of the collected leaves reached nearly 7.5 percent, then crushed and stored in a dry area until analysis. While the Moringa oleifera cake seeds (MOCS) were obtained from the farm of the National Research Center in Nubaria.

### Blood samples:

The first blood sample was collected after 15 days of the starting day of the present study. Blood samples were collected biweekly from the jugular vein of each ram in the morning before feeding or drinking with a disposable syringe, delivered in sterile tubes containing K3-EDTA (1 mg/ml) to prevent coagulation, and transported to the laboratory for blood analysis.

A portion of the blood sample was used to determine the total leukocyte count (WBC, x103/mm3), as well as differential leukocytes using a hematology analyser (Sysmex KX-21N Auto Hematology Analyzer, KOBE, JAPAN. The other portion of the blood sample was centrifuged at 3000 rpm for 15 min to obtain

clear plasma, and immediately held at -20 °C until the estimation of malondialdehyde (MDA), total antioxidant capacity (TAC), triglyceride (TG) as well as types of serum immunoglobulins (IgG & IgM).

Plasma TG concentrations (mg/dl) were measured calorimetrically using commercial kits (Biosystems S.A. Costa Brava, Barcelona, Spain). Reactivos GPL, Barcelona, Spain, was used to examine the activity of MDA and TAC in blood plasma and serum immunoglobulin types (IgG) and M (IgM) were determined using the ELISA technique as (mg/dl) or antibodies.

### Quantitative antibody test:

Each ram was injected intravenously with 0.1 ml of 2.5% Chicken Red Blood Cells (CRBC) solution in physiological saline (0.9% NaCl). Then, the immune serum was collected 7 days and 14 days after immunization, the antibody titer was estimated using the hemagglutinating test assay as described by Siegel and Gros, (1980).

### Statistical analysis:

All data were statistically analyzed using least squares procedure described by SAS (2007). Through this software, Duncan Multiple Range Test was applied to test the level of significant among the means. The significant level was set as $P<0.05$.

## RESULTS AND DISCUSSION

### I- Effect of MOL and MOSC supplementation in rations of Barki rams on antibody response.

The results of the quantitative antibody test (Table, 1) showed that before immunization there were no significant differences between the three different experimental groups in the hemagglutinin produced by the rams. While after 7 days of immunization, the mean antibody titer in the blood of rams fed with Moring seeds was significantly ($P < 0.05$) higher (1.81 ±0.5) than that in the blood of the group of rams fed on rations containing Moringa leaves (0.54 ± 0.5) or those produced in the blood of the rams fed on moringa-free rations (0.42 ± 0.3), the difference between the last two groups was not significant. After 14 days of immunization, the group of rams supplemented with moringa seeds achieved the highest antibody titer production (4.82 ± 1.16), followed by that produced in the blood of rams in the control group (3.25 ± 1.29), however the lowest production score was recorded for rams fed on rations containing Moringa leaves ($2.41^{a}$±1.2) with no significant differences among the three experimental groups.

In addition, the results presented in Table (1) indicated that after two weeks of immunization the level of antibodies in the blood of the three experimental groups of rams under consideration increased significantly ($P < 0.05$) compared to the level recorded before or a week after immunization. This increase during this period ranged from 181% to 703% compared to that recorded before immunization. The highest increase in antibodies after two weeks of immunization was recorded in the blood of rams fed Moringa seeds (703%) and the lowest one (181%) in the blood of rams fed with Moringa leaves. The rate of antibodies produced in the blood of rams fed Moringa-free increased by 577%. In other words, results pointed that supplementation rams with Moring seeds resulted an increase in the level of antibodies in blood after 7 or 14 days of immunization as compared to that produced in the blood of rams supplemented with Moringa leaves or that fed without Moringa supplements.

**Table (1): Effect of MOL and MOSC supplementation in rations of Barki rams on the quantitative antibody response:**

| Experimental groups | Before | After 7 days | After 14 days |
|---|---|---|---|
| G1(*MOL)* | 0.60,A,b±0.1 | $0.54^{B,b}$±0.5 | $2.41^{A,a}$±1.2 |
| G2(*MOSC)* | 0.60A,c±0.1 | $1.81^{A,b}$,±0.5 | $4.82^{A,a}$±1.2 |
| G3(control) | 0.48A,b±0.1 | $0.42^{B,b}$±0.3 | $3.25^{A,a}$±1.3 |

**A,B:** Values in the same **column** with different super script are significantly differed ($p < 0.05$)
**a,b**: Values in the same **row** within different super script are significantly differed ($p < 0.05$)
***MOL*: *moringa olifera leaves*** ***MOSC:*** ***moringa olifera seed cake.***

From these results, it can be concluded that:

**First**: all examined animals in the three experimental groups were able to establish a significant immunization response after a single intravenous injection of CRBC. Similar results were reported by Hosseinzade et al., (2019) and Nfambi et al., (2015) who observed that the ethanolic extract of Moringa oleifera enhanced the response of sheep red blood cell (SRBC) antibodies in mice.

**Second**: Adding Moringa seeds, to a ram's diet can increase the production of antibodies in the blood.

## II- Effect of MOL and MOSC supplementation in rations of Barki rams on total antioxidant activity (TAC), Malondialdehyde (MAD), immunoglobulin (IgG, IgM.)

The result presented in Table (2) indicates that adding Moringa leaves or Moringa seeds to the ram's ration significantly affected the levels of MAD and insignificantly TAC, IgM and IgG levels.

In this regard, the addition of Moringa leaves or seeds to the ration of rams increased the TAC activity in the blood of the rams group fed a ration containing moringa seeds (8.88 ± 1.7) or moringa leaves (10.08±1.7) compared to those rams fed a ration free of Moringa (7.30 ± 2.0) without statistically significant differences as shown in Table (2). Conversely, the addition of Moringa leaves or seeds resulted in a significant decrease in MAD activity in the group of rams fed a ration containing Moringa seeds (3.05 ± 2.0) or Moringa leaves (3.74 ± 2.0) compared to those of rams fed Moringa - Free ration (19.78 ± 2.2).

Our results are consistent with those achieved in rabbits by El-Gindy et al. 2017 and EL-Badawi et al., 2016 who showed significant improvement in serum TAC in rabbits fed diets containing Moringa or those results of Oseni and Idowu 2014 who reported that Moringa leaves (MOL) or MOL extract increased TAC, antioxidant enzymes such as GSH, GSH-Px and catalase but decreased the concentration of MDA in the blood of mice. In addition, Lamo et al., 2016 indicated that adding aqueous Moringa extract at a concentration of 100, 200 or 400 mg/kg to the diet of rats improved the activity of antioxidant enzymes and reduced the concentration of MDA.

This decrease in MDA concentration and increase in TAC in blood samples of animals fed a diet containing Moringa leaves or seeds compared to those animals fed a moringa-free diet may be related to reduced fat deposition through decreasing the activities of malate dehydrogenase and lipoprotein lipase or increasing the hormone-sensitive lipase activity in the adipose tissue (Mbikay, 2012 & Lu et al., 2007 ) or it may be attributed to the presence of flavonoids that reduce oxidative stress (salem et al., 2020).

**Table (2): Effect of MOL and MOSC supplementation in rations of Barki rams on total antioxidant activity (TAC), Malondialdehyde (MAD), immunoglobulin (IgG, IgM.) (mean ±SE).**

| Measurements | | Experimental groups | | |
|---|---|---|---|---|
| | | G1(***MOL)*** | G2(***MOSC)*** | G3(**control**) |
| TAC | | $10.08^{a}$±1.7 | $8.88^{a}$±1.7 | $7.30^{a}$±2.0 |
| MAD | | $3.74^{b}$±2.0 | $3.05^{b}$±2.0 | $19.78^{a}$±2.2 |
| (Igs) | IgG | $554.80^{a}$±111.2 | $493.40^{a}$±111.2 | $458.90^{a}$±131.2 |
| | IgM | $225.10^{a}$±78.4 | $413.20^{a}$±78.4 | $314.00^{a}$±88.9 |

**a,b** : Means within each row within certain measurement with different superscript are significant (P<0.05).
***MOL*****:** *moringa olifera leaves* ***MOSC:*** *moringa olifera seed cake.*
**TAC= Total** antioxidant, **MAD :** Malondialdehyde, **Igs**: Immunoglobulins, IgG: Immunoglobulin
**IgM**= Immunoglobulin **M**.

In addition, as shown in Table (2) neither supplementation of moringa leaves nor moringa seeds into rams' ration affected immunoglobulin IgG and IgM levels significantly. However, Salem et al., 2020 reported that immunoglobulin G (IgG) and IgM were affected by MOL supplementation and the degree of effect was related to the percentage of addition or presence of MOL in rabbit diets where IgG was increased by MOL supplementation, the highest increase (2.6% above control) was observed under MOL20, while MOL10 increased IgG by (0.7%) compared to control, MOL30 reduced IgG to reach (2.7%) under control.

As well as, the results of the present study showed that the highest level of immunoglobulin G was recorded in blood samples of rams fed diet containing Moringa leaves (554.80±111.2), followed by that measured in the blood of rams fed ration supplemented with Moringa seeds (493.40±111.2) and the lowest one was for rams fed a Moringa-free ration (458.90 ± 131.2) with no statistically significant differences. However, the highest level of immunoglobulin M was recorded in blood samples of rams fed diet containing Moringa seeds (413.20±78.4), followed by that measured in the blood of rams fed a Moringa-free ration (314.00±88.9) and the lowest one was for rams fed ration supplemented with Moringa leaves (225.10±78.4) with no statistically significant differences.

Previous research reports concluded that M. oleifera is an immunomodulatory agent that can stimulate the production of IgG, IgM and IgA (Al-Badawi et al., 2016, El Gendy et al., 2017, Al-Majali et al., 2017 and Abd-Elhakim et al., 2018) as well as the aqueous extract of M. oleifera showed a strong immune-modulating effect that could modulate B-cell activation and stimulate the production of IgM, IgA and IgG level after administration (Ojeka et al. 2016), and T cells where it will result for induction of IL 10 production (Fard et al. 2015; Tan et al. 2015) and inhibit production of TNFα, IL-6, IL-8 (Kooltheat et al. 2014) and IL-2 (Sashidhara et al. 2009).

### III- Effect of MOL and MOSC supplementation in rations of Barki rams on WBC count and leukocyte differentiation.

The data in Table (3) illustrate the effect of feeding adult Barki rams on rations including MOL and MOSC on White blood cell count as well as leukocyte differentiation (lymphocytes, lymphocytes, eosinophils, basophils, and neutrophils).

It is evident that there are no significant differences between these treatments in the total number of white blood cells or any relevant differentiation of leukocytes except for monocytes (Table, 3). In this regard, supplementation of Moringa seeds (MOSC) or leaves (MOL) slightly increased the total count of WBC (8.33±5.86 and 8.00±5.86, respectively) compared to those recorded in the control group rams (7.80±6.77). Similar results were obtained in rabbits (Jiwuba et al., 2016, El-Badawi et al. al., 2016, El-Gindy et al., 2017 & salem et al., 2020) and in rats (Adedapo et al., 2005, Gupta et al., 2010, Isitua & Ibeh, 2013 and Asomugha et al., 2015) but the increase in WBC count was stastically significant. Also, Al-Majali et al. (2017) showed that supplementation of ethanolic Moringa peregrina extract to rats resulted in a significant increase in total WBC counts in peripheral blood. Lamou et al., 2016, Kasolo et al., 2010 attributed the increase in WBCs to decreased oligosaccharides in Moringa oleifera leaves. On the other side, the obtained data showed that the addition of Moringa seeds to rams' diets resulted in a lower percentage of eosinophilic cells (1.3 ± 0.24) compared to those obtained in blood samples of rams fed with moringa leaves (1.5 ± 0.21) or those of the control group (1.5 ± 0.21), the differences between the three experimental groups were not significant.

**Table (3): Effect of MOL and MOSC supplementation in rations of Barki rams on WBC count and leukocyte differentiation (mean ±SE).**

| Experimental groups | WBCs (103/m) | Lymphocytes (%) | Monocytes (%) | Eosinophils (%) | Basophils (%) | Neutrophils (%) |
|---|---|---|---|---|---|---|
| G1(*MOL)* | 8.00±5.86 | 45.00±3.32 | 2.75±0.37$^{b}$ | 1.5 ±0.21 | 0.00±0.03 | 51.25±3.51 |
| G2(*MOSC)* | 8.33±5.86 | 43.33±3.84 | 3.00±0.43$^{a}$ | 1.3 ±0.24 | 0.00±0.04 | 52.67±4.05 |
| G3(control) | 7.80±6.77 | 51.25±3.32 | 2.25±0.37$^{b}$ | 1.5 ±0.21 | 0.00±0.03 | 45.00±3.51 |

* Means within each column not sharing superscript are significant (P<0.05).
**MO**L: moringa olif *era leaves, **MOSC**: moringa oleifera seed cake**, WBC**: white blood cells

The results of the current study (Table, 3) also showed that the neutrophil cells in the blood of rams fed with Moringa seeds or leaves (51.25±3.51 and 51.25±3.51, respectively) were higher than those in the blood of rams fed a basic diet without supplements (45.00±3.51) in agreement with the results reported by Gupta et al., 2010 and disagreeing with the results of Salem et al., 2020. On the contrary, the addition of Moringa seeds or leaves resulted in a slight decrease in the total number of lymphocytes (43.33 ± 3.84 and 45.00 ± 3.32, respectively) compared to the control group (65.71 ± 5.57).

It could be concluded that feeding Barki rams forage supplemented with Moringa seeds resulted in a slight increase in the total number of white blood cells as well as the proportion neutrophils compared to that recorded in rams fed with Moringa leaves or without Moringa supplement (Table, 3). In this regard, Duke (1977) stated that many cells and organs work together to protect the body. White blood cells (leukocytes) play an important role in the immune system as well as neutrophils which are the first responder of the innate immune system, along with other cells of granulocytes (eosinophils and monocytes. *This may mean that adding Moringa and especially Moringa seeds to sheep rations can boost the ram's immunity.*

## IV- Effect of MOL and MOSC supplementation in rations of Barki rams on the activity of serum transaminases (GOT, GPT) and kidney function (creatinin and urea) in blood of Barki rams

The results in Table (4) show that kidney function, serum transaminase concentrations and level in Barki rams were affected in different ways by adding MOL or MOSC to rams' feed.

### a- Kidney function:

Table (4) shows that the kidney function of the Barki rams was significantly (P<0.05) affected by feeding ration contained leaves or seeds of Moringa. In this regard, results in table (4) indicated that supplementation of rams with MOSC resulted in significantly lower serum creatinin values (0.97±0.08 mg/dl) compared to either rams supplemented with MOL (1.58±0.08) or those fed a moringa-free diet (1.42±0.09), while the concentration of Uric acid recorded in the blood samples of rams fed MOL was significantly (P<0.05) lower (4.05±0.26 mg/dl) than that in those of rams fed MOSC (5.19±0.26 mg/dl) or those of control group rams (4.36±0.29).

### b- Transaminases:

Concerning to the effect of feeding Barki rams ration supplemented with MOl or MOSC on the activity of transaminases (GOT & GPT), data listed in Table (4) illustrate that the activity of GOT was significantly (P<0.05) and GPT insignificantly affected. The GOT concentration was significantly lower in the blood of rams fed with MOl (50.50 ± 13.33 u/L) compared to the concentration estimated in the blood of control group rams fed without any Moringa supplement (69.06±14.90 u/L), and insignificantly in the blood samples of rams fed with MOSC (59.62 ± 13.33 u/L). While, the lowest level of GPT was recorded in the blood of rams supplemented with MOSC (38.88± 11.98 u/L) compared to that of rams supplemented with MOL (48.04 ± t11.98 u/L) or rams of the control group (56.66± 13.40 u/L).

**Table (4): Effect of MOL and MOSC supplementation in rations of Barki rams on the activity of serum transaminases (GOT, GPT), kidney function (creatinin and urea) in blood of Barki rams (mean ±SE).**

| Measurements | | Supplementation treatments | | |
|---|---|---|---|---|
| | | G1(MOL) | G2(MOSC) | G3(control) |
| Kidney function | Creatinin (mg/dl) | $1.58 \pm 0.08^{a}$ | $0.97 \pm 0.08^{c}$ | $1.42 \pm 0.09^{ab}$ |
| | Uric acid (mg/dl) | $4.05 \pm 0.26^{bc}$ | $5.19 \pm 0.26^{a}$ | $4.36 \pm 0.29^{ab}$ |
| Transaminases | GOT (u/L) | $50.50 \pm 13.33^{b}$ | $59.62 \pm 13.33^{ab}$ | $69.06 \pm 14.90^{a}$ |
| | GPT (u/L) | 48.04±11.98 | 38.88±11.98 | 56.66±13.40 |

**a,b** : Means within each row within certain measurement with different superscript are significant (P<0.05).
**MOL:** moringa olifera leaves, **MOSC:** moringa oleifera seed cake, **GOT:** glutamic oxaloacetic transaminase, **GPT:** glutamic pyruvic transaminase

The results regarding the effect of supplementing Barki rams' diets with MOL or MOSC on kidney function and the activities of transaminases concluded that the activity of GPT was the lower for the groups fed diet contained MOSC (38.88 u/L), and those fed diet contained MOL (48.04 u/L), meanwhile greater values (56.66 u/L) were recorded by the group fed free Moringa-supplemented diet. To some extent the same results were recorded for the GOT where the lower activity was recorded in blood samples of rams fed with MOL (50.50 u/L) and those fed with MOSC (59.62 u/L) without significant difference meanwhile the highest activity (69.06 u/L) was measured for the group fed free Moringa-supplemented diet. These results may indicate that supplementation of rams with MOSC tends to attenuate or mild hepatic and renal necrosis.

The results of El-Hak et al., 2018 reported a positive effect of M. seeds on liver enzymes and structure at all doses they used (500, 1000 and 2000 mg/kg body weight) as there was a significant decrease in ALT and AST when compared to the control group. On the other side, Nguyen-Lefebvre & Horuzsko, (2015) indicated that the leaves of Moringa oleifera and its cake seeds protect hepatic tissues to some extent from degenerative and inflammatory factors, as Moringa leaves activate Kupffer cells, which are hepatocytes that play an important role in maintaining liver function and as well as they are macrophages that have an important function in the innate immune response of the liver. This might be due to the presence of saponins, flavonoids and phenolics (Paliwal et al., 2011 & Sharma et al., 2011).

## Conclusion

Addition of Moringa oleifera supplements (leaves or seeds) especially seeds (MOSC) to the diet of Barki rams can increase antibody production, total white blood cell count, neutrophil count, TAC and decrease MAD, in addition to positive effect on kidney function and liver enzymes, MOSC enhances ram's immunity.

## REFERENCES

Abd-Elhakim, Y. M.; El Bohi, K. M.; Hassan, S. K.; El Sayed, S. and Abd-Elmotal, S. M. (2018). Palliative effects of Moringa olifera ethanolic extract on hemato-immunologic impacts of melamine in rats. *F*ood and Chemical Toxicology, 114: 1-10

Adedapo, A.A.; Abatan, M.O.; Idowu, S.O.; et al. (2005): Toxic effects of chromatographic fractions of Phyllanthus amarus on the serum biochemistry of rats. Phytother Res 19: 812-815.

Al-Majali, I. S.; Al-Oran, S. A.; Hassuneh, M. R.; Al-Qaralleh, H. N.; Rayyan, W. A.; Al-Thunibat, O. Y.; Mallah, E.; Abu-Rayyan, A. and Salem, S. (2017). Immunomodulatory effect of Moringa peregrina leaves, ex vivo and in vivo study. Central-European journal of immunology, 42(3): 231–238. https://doi.org/10.5114/ceji.2017.70964.

Anwar, F.; Latif, S.; Ashraf, M. and Gilani, A. H. (2007). Moringa oleifera: a food plant with multiple medicinal uses. Phytotherapy

Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 21(1): 17-25.

Asomugha, A. L., Ezejindu, D. N., Asomugha, R. N., Anyabolu, A. E., & Ojukwu, P. C. (2015). Evaluation of toxicity effect of graded doses of Moringa oleifera leaf extract on blood indices using 20 adult Wistar rats. Int J Biom Adv Res, 6(2): 98-102. doi: 10.7439/ijbar.v6i2.1535.

Baldisserotto, A.; Buso, P.; Radice, M.; Dissette, V.; Lampronti, I.; Gambari, R. and Vertuani, S. (2018). Moringa oleifera leaf extracts as multifunctional ingredients for "natural and organic" sunscreens and photoprotective preparations. Molecules, 23(3): 664.

Bannam, T. L.; Yan, X. X.; Harrison, P. F.; Seemann, T.; Keyburn, A. L.; Stubenrauch, C. and Rood, J. I. (2011). Necrotic enteritis-derived Clostridium perfringens strain with three closely related independently conjugative toxin and antibiotic resistance plasmids. MBio, 2(5): e00190-11.

Bedi, O. K. R. V. Bijjem, Kumar, P. and Gauttam, V. (2016). "Herbal induced hepatoprotection and hepatotoxicity: a critical review," Indian Journal of Physiology and Pharmacology, 60 (1): 6–21.

Bichi, M. H. (2013). A review of the applications of Moringa oleifera seeds extract in water treatment. Civil and Environmental Research, 3(8): 1-10.

Cuellar-Nuñez, M. L.; Luzardo-Ocampo, I.; Campos-Vega, R.; Gallegos-Corona, M. A.; De Mejía, E. G. and Loarca-Piña, G. (2018). Physicochemical and nutraceutical properties of moringa (Moringa oleifera) leaves and their effects in an in vivo AOM/DSS-induced colorectal carcinogenesis model. Food Research International, 105: 159-168.

Dhakad, A. K.; Ikram, M.; Sharma, S.; Khan, S.; Pandey, V. V. and Singh, A. (2019). Biological, nutritional, and therapeutic significance of Moringa oleifera Lam. Phytotherapy Research, 33(11): 2870-2903.

Duke H.H. (1977) Dukes' Physiology of domestic animals, Comstock Pub. Associates- 9th ed.

El-Badawi, A. Y.; El-Wardany, I.; Abedo, A. A. and Omer, H. A. A. (2016). Haematological, blood biochemical constituents and histopathological responses of growing rabbits fed different levels of moringa leaves. International Journal of Chemistry Technology Research. ;9; 111-21.

El-Gindy, Y. M.; Zewell, H. S. and Hamad, M. (2017). Effect of Moringa leaf as a natural antioxidant on growth performance, blood lipid profile and immune response of rabbits under moderate heat stress. Egypt. *J. Poult. Sci*, *37*(2), 333-344.

EL-Hak, H.N.G.; Abdel Raouf A. Moustafa., and Samira R. Mansour, S.R. (2018). Toxic effect of Moringa peregrina seeds on histological and biochemical analyses of adult male Albino rats. Toxicol Rep. 5: 38–45 .

Fahey, J. W. (2005). Moringa oleifera: a review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. Trees for life Journal, 1(5): 1-15.

Fard, M. T.; Arulselvan, P.; Govindarajan Karthivashan, S. K. A. and Fakurazi, S. (2015). Bioactive extract from Moringa oleifera inhibits the pro-inflammatory mediators in lipopolysaccharide stimulated macrophages. *Pharmacognosy magazine*, *11*(Suppl 4), S556.

Farouk, A. E. A.; Ghouse, F. A. H. and Ridzwan, B. H. (2007). New bacterial species isolated from Malaysian sea cucumbers with optimized secreted antibacterial activity. American Journal of Biochemistry and Biotechnology, 3(2): 60-65.

Gholamiandehkordi, A.; Eeckhaut, V.; Lanckriet, A.; Timbermont, L.; Bjerrum, L.; Ducatelle, R. and Van Immerseel, F. (2009). Antimicrobial resistance in Clostridium perfringens isolates from broilers in Belgium. Veterinary research communications, 33(8): 1031-1037.

Gupta, A.; Gautam, M. K.; Singh, R. K.; Kumar, M. V.; Rao, C. V.; Goel, R. K. and

Anupurba, S. (2010). Immunomodulatory effect of Moringa oleifera Lam. extract on cyclophosphamide induced toxicity in mice. Indian Journal of Experimental Biology, 48, 11, 1157–1160.

Hao, H.; Cheng, G.; Iqbal, Z.; Ai, X.; Hussain, H.I.; Huang, L.; Dai, M.; Wang, Y.; Liu, Z. and Yuan, Z. (2014). Benefits and risks of antimicrobial use in food-producing animals. Front Microbiol.;5:288.

Hosseinzade, A.; Sadeghi, O.; Naghdipour Biregani, A.; Soukhtehzari, S.; Brandt, G. S. and Esmaillzadeh, A. (2019). Immunomodulatory effects of flavonoids: possible induction of T CD4+ regulatory cells through suppression of mTOR pathway signaling activity. Frontiers in immunology, 51.

Isitua, C. C. and Ibeh, I. N. (2013). Toxicological assessment of aqueous extract of Moringa oleifera and Caulis bambusae leaves in rabbits. Journal of Clinical Toxicology S, 12, 4. doi:10.4172/2161-

Jaiswal, D.; Rai, P. K.; Kumar, A.; Mehta, S. and Watal, G. (2009). Effect of Moringa oleifera Lam. leaves aqueous extract therapy on hyperglycemic rats. Journal of ethnopharmacology, 123(3): 392-396. doi: 10.1016/j.jep.2009.03.036.

Jiwuba, P. C.; Ikwunze, K.; Dauda, E. and Ugwu, D. O. (2016). Haematological and serum biochemical indices of growing rabbits fed diets containing varying levels of Moringa oleifera leaf meal. British Biotechnology Journal, 15(2): 1-7.

Kasolo, J. N.; Bimenya, G. S.; Ojok, L.; Ochieng, J. and Ogwal-Okeng, J. W. (2010). Phytochemicals and uses of Moringa oleifera leaves in Ugandan rural communities. Journal of Medicinal Plants Research, 4(9): 753-757.

Kooltheat, N.; Sranujit, R. P.; Chumark, P.; Potup, P.; Laytragoon-Lewin, N. and Usuwanthim, K. (2014). An ethyl acetate fraction of Moringa oleifera Lam. inhibits human macrophage cytokine production induced by cigarette smoke. Nutrients, 6(2): 697-710.

Lamou, B.; Taiwe, G. S.; Hamadou, A.; Houlray, J.; Atour, M. M. and Tan, P. V. (2016). Antioxidant and antifatigue properties of the aqueous extract of Moringa oleifera in rats subjected to forced swimming endurance test. Oxidative medicine and cellular longevity, ; 1-9. doi.org/10.1155/2016/3517824.

Liang, L.; Wang, C.; Li, S.; Chu, X. and Sun, K. (2019). Nutritional compositions of Indian Moringa oleifera seed and antioxidant activity of its polypeptides. Food science & nutrition, 7(5): 1754-1760.

Lu, P.; Rangan, A.; Chan, S. Y.; Appling, D. R.; Hoffman, D. W. and Marcotte, E. M. (2007). Global metabolic changes following loss of a feedback loop reveal dynamic steady states of the yeast metabolome. Metabolic engineering, 9(1): 8-20.

Mahanta, J. D.; Borgohain, B.; Sarma, M.; Sapcota, D. and Hussain, J. (2017). Effect of dietary supplementation of herbal growth promoter on performance of commercial broiler chicken. Indian Journal of Animal Research, 51(6): 1097-1100. doi: 10.18805/ijar.11420.

Mahfuz, S. and Piao, X. S. (2019). Application of Moringa (Moringa oleifera) as natural feed supplement in poultry diets. Animals, 9(7): 431.

Mbikay, M. (2012). Therapeutic potential of Moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: A review. Front Pharmacol. 3: 24.

Nfambi, J.; Bbosa, G. S.; Sembajwe, L. F.; Gakunga, J. and Kasolo, J. N. (2015). Immunomodulatory activity of methanolic leaf extract of Moringa oleifera in Wistar albino rats. Journal of basic and clinical physiology and pharmacology, 26(6): 603-611.

Nguyen-Lefebvre, A.H. and Anatolij, H. (2015) Kupffer Cell Metabolism and Function. J Enzymol Metab, 1(1): 101.

NRC, (1985). National Research Council. Nutrient requirements of sheep (Vol. 5). National Academies Press

Ojeka, S. O., Obia, O., & Dapper, D. V. (2016). Effect of acute administration of aqueous leaf extract of Moringa oleifera on immunoglobulin levels in Wistar Rats. European Journal of Medicinal Plants, 14(4): 1-7. https://doi.org/10.9734/EJMP/2016/24880

Olson, M. E. (2002). Combining data from DNA sequences and morphology for a phylogeny of Moringaceae (Brassicales). Systematic Botany, 27(1): 55-73.

Oseni, O. A. and Idowu, A. S. K. (2014). Inhibitory activity of aqueous extracts of horseradiash Moringa oleifera (Lam) and nutmeg Myristica fragrans (Houtt) on Oxidative stress in alloxan induced diabetic male Wistar albino rats. Am J Biochem Mol Biol, 4(2): 64-75.

Pakade, V.E.; E. Cukrowska and Chimuka, L. (2013). Comparison of antioxidant activity of Moringa oleifera and selected vegetables in South Africa. South Afr. J. Sci., 109 (314): 1154-1158.

Palada, M.C. and Chang, LC. (2003). Suggested cultivation practices for Moringa. AVRDC Publication #03-545. AVRDC [Cited 2015 Nov 25]. Available at: http://www.avrdc.org/LC/ indigenous/moringa.pdf.

Paliwal, R.; Sharma, V. and Pracheta (2011). A review on horse radish tree (Moringa oleifera): A multipurpose tree with high economic and commercial importance. Asian J. Biotechnol.; 3(4): 317-328.

Salem, M. I.; El-Sebai, A.; Elnagar, S. A.; El-hady, A. and Mohamed, A. (2020). Evaluation of lipid profile, antioxidant and immunity statuses of rabbits fed Moringa oleifera leaves. *Asian-Australasian Journal of Animal Sciences*

Sashidhara, K.V.; Rosaiah, J.N.; Tyagi, E.; Shukla, R.; Raghubir, R.; Rajendran, S.M. (2009). Rare dipeptide and urea derivatives from roots of Moringa oleifera as potential anti-inflammatory and antinociceptive agents. Eur J Med Chem 44: 432–436.

Sharma, V.; Paliwal, R.; Sharma, P. and Sharma, S. (2011). Phytochemical analysis and evaluation of antioxidant activities of hydro-ethanolic extracts of Moringa oleifera Lam. pods. J. Pharm. Res, 4(2): 554-557.

Siegel, P. B. and Gross, W. B. (1980). Production and persistence of antibodies in chickens to sheep erythrocytes. 1. Directional selection. Poultry Science, 59(1), 1-5.

Singh, V. P.; Arulanantham, A.; Parisipogula, V.; Arulanantham, S. and Biswas, A. (2018). Moringa olifera: nutrient dense food source and world's most useful plant to ensure nutritional security, good health and eradication of malnutrition. European Journal of Nutrition & Food Safety, 8(4): 204-214.

Tan, W. S.; Arulselvan, P.; Karthivashan, G. and Fakurazi, S. (2015). Moringa oleifera flower extract suppresses the activation of inflammatory mediators in lipopolysaccharide-stimulated RAW 264.7 macrophages via NF-κB pathway. *M*ediators of inflammation, 2015.

Wang, Z.; Qi, F.; Cui, Y.; Zhao, L.; Sun, X.; Tang, W. and Cai, P. (2018). An update on Chinese herbal medicines as adjuvant treatment of anticancer therapeutics. Bioscience Trends, 12(3): 220-239.

Zidaric, V.; Pardon, B.; Dos Vultos, T.; Deprez, P.; Brouwer, M. S. M.; Roberts, A. P. and Rupnik, M. (2012). Different antibiotic resistance and sporulation properties within multiclonal Clostridium difficile PCR ribotypes 078, 126, and 033 in a single calf farm. Applied and environmental microbiology, 78(24): 8515-8522.

# تأثير مكملات المورينجا اوليفيرا على الاستجابة المناعية فى الاغنام البرقى

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## الملخص العربى

تم استخدام خمسة عشر كباش برقي بالغًا لدراسة تأثير مكملات MOL أو MOSC في الحصص الغذائية على الاستجابة المناعية (IR) ، إجمالي سعة مضادات الأكسدة (TAC، MAD)) ، الغلوبولين المناعي ( IgG، IgM) وكذلك عدد خلايا الدم البيضاء و تمايزها كما تم تقديرنشاط إنزيمات الكبد ووظيفة الكلي. تم تقسيم الكباش إلى ٣ مجموعات متشابهة ، تم تغذية الكباش في G1 بنظام غذائي أساسي مكمل بـ MOL بتركيز ٠,٥٪ من العليقة المركزه / يوم ، بينما تم تغذية الكباش في G2 بنظام غذائي أساسي مكمل بـ MOSC بتركيز ١٠٪ من العليقة المركزه / يوم بينما تم تغذية G3 بنظام غذائي أساسي بدون مكملات. تم جمع عينات الدم كل أسبوعين من كل كبش وتم تقدير عدد كريات الدم البيضاء وتقدير كل من MDA، TAC، IgG، IgM ونشاط الترانساميناسات المصل وكذلك تقييم وظائف الكلى. وتم قياس استجابة الجسم المضاد (IR) أيضًا عن طريق تطبيق اختبار الجسم المضاد الكمي. أشارت النتائج إلى أن مكملات الكباش مع MOSC زادت في مستوى الأجسام المضادة في دم الكبش بعد ٧ أو ١٤ يومًا من التحصين مقارنة بتلك المنتجة في دم الكباش المكملة بـ MOL أو التي يتم تغذيتها بدون مكملات ، بالإضافة إلى زيادة TAC وانخفاض MAD في عينات الدم ، فقد إرتفع نشاط TAC في دم مجموعة الكباش التي تم تغذيتها على حصة تحتوي على MOSC أو MOL بدرجة قليلة مقارنة بتلك الكباش التي تم تغذيتها على عليقة خالية من المورينجا و العكس من ذلك ، كان نشاط MAD في مجموعة الكباش التي تم تغذيتها على حصة تحتوي على MOSC 0) أو MOL أقل بكثير مقارنة بالكباش التي تتغذي عليقة خالية من المورينجا . تأثرت مستويات IgG و IgM بشكل مختلف نتيجة تغذية الكباش بأوراق أو بذور المورينجا. تم تسجيل أعلى مستوى من IgG في عينات الدم من الكباش التي تم تغذيتها على MOl تليها تلك التي تم قياسها في دم الكباش التي تتغذي على MOSC وأقلها كانت للكباش التي تم تغذيتها على عليقة خالية من المورينجا . ومع ذلك ، تم تسجيل أعلى مستوى من IgM في عينات الدم من الكباش التي تم تغذيتها على النظام الغذائي المحتوي على MOSC ، تلاها تلك التي تم قياسها في دم الكباش التي تم تغذيتها على عليقة خالية من المورينجا وأقلها كانت للكباش التى تغذت على MOL مع عدم وجود فروق ذات دلالة إحصائية. أدت مكملات MOSC أو MOL فى علائق كباش البرقى إلى زيادة طفيفة في العدد الإجمالي لخلايا كريات الدم البيضاء (٨,٣٣ ± ٥,٨٦ و ٨,٠٠ ± ٥,٨٦) وخلايا النيتروفيل ((٥٢,٦٧ ± ٣,٥١ و ٥١,٢٥ ± ٣,٥١) مقارنة بتلك الموجودة في مجموعة الكباش الضابطة (٧,٨٠ ± ٦,٧٧ و ٤٥,٠٠ ± ٣,٥١ ، على التوالي) ، ومع ذلك ، فإن مكملات الكباش مع MOSC قللت بشكل كبير من الكرياتينين (٠,٩٧ ± ٠,٠٨ مجم / ديسيلتر) و GPT (38.88 ± 11.98 ش / لتر) مقارنة بتلك المكملة ب MOL- 1.58) 0.08 ± مجم / ديسيلتر ، ٤٨,٠٤ ± 98. t11 ش / لتر) أو المجموعة الضابطة (١,٤٢ ± ٠,٠٩ مجم / ديسيلتر و ٥٦,٦٦ ± ١٣,٤٠ ش / لتر). ويستنتج من هذه الدراسة أن إضافة مكملات المورينكا خاصة بذورها (MOSC) إلى العليقة الغذائية لكباش البرقي يمكن أن تزيد من إنتاج الأجسام المضادة ، وإجمالي عدد خلايا الدم البيضاء ، وعدد العدلات ، و TAC وانخفاض MAD ، بالإضافة إلى التأثير الإيجابي على وظائف الكلى وإنزيمات الكبد ويعزز من مناعة الكباش.