*J. Animal and Poultry Prod., Mansoura Univ., Vol.8 (9): 367- 379 , 2017*

# Impact of Dietary Moringa Oleifera Leaves Supplementation on Semen Characteristics, Oxidative Stress, Physiological Response and Blood Parameters of Heat Stressed Buffalo Bulls

**Wafa, W. M.[1]; H. A. El-Nagar[1]; A. A. Gabr[1] and M. M. Rezk[2]**

**[1] Animal Production Research Institute (APRI), Ministry of Agriculture, Dokki, Giza, Egypt.**

**[2] Isotopes Department, Nuclear Materials Authority, Cairo, Egypt.**



## ABSTRACT

This study aimed to evaluate dietary supplementation of Moringa oleifera leaves (MOL) on semen quality, oxidative stress, thermal regulation and health status of heat stressed buffalo bulls. Eighteen sexually mature Egyptian buffalo bulls were divided into three groups, 6 in each. Bulls in G1 were fed ration composed of concentrate fed mixture (CFM), berseem hay and rice straw (control). Bulls in G2 and G3 were fed the same CFM supplemented with MOL at levels of 4 and 8% of CFM, respectively for one month pre-semen collection and 4 months as semen collection period. Semen was collected twice weekly and evaluated for percentages of individual motility (IM), livability (SL), abnormality (SA) and damaged acrosome (DA) of sperm cells. Response of spermatozoa to hypo-osmotic test (percentage of curled spermatozoa) at 50 mOsm/l for 30 min was also recorded. Rectal (RT) and skin (ST) temperatures, respiration rate (RR) and pulse rate (PR) were recorded. Blood samples were taken pre-treatment and during 1st, 2nd, 3rd and 4th months of semen collection to determine hemoglobin concentration (Hb), packed cell volume (PCV%), count of red (RBCs) and white (WBCs) blood cells. Concentration of total proteins (TP), albumin (AL), globulin (GL), glucose (GLU), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), urea, uric acid, creatinine (Cr) and testosterone, as well activity of AST, ALT, superoxide dismutase (SOD), catalase and glutathione (GSH) were determined in blood serum. Concentration of TG and TC, and activity of AST, ALT, SOD, catalase and GSH were estimated also in seminal plasma. Results showed that RT and ST, RR and PR decreased (P<0.05) in G3 than in G2 and G1. Each of RT and ST, RR and PR increased (P<0.05) up to 3rd collection month, then decreased at the 4th collection month in association with THI values. Percentage of IM, SL, SA, CT and DA were improved (P<0.05) in G2 and G3 as compared to G1, being the best (P<0.05) in G3. All previous parameters were improved (P<0.05) by advancing collection month. Both TC and TG in seminal plasma decreased (P<0.05) in G3 as compared to G1 and G2. Activity of AST and ALT decreased (P<0.05), while catalase, GSH and SOD activities increased (P<0.05) in seminal plasma of G2 and G3 as compared to G1. Each of TC, ALT, catalase, GSH and SOD in seminal plasma increased (P<0.05), while TG decreased (P<0.05) by advancing collection month, while AST was not affected. Serum testosterone concentration was higher (P<0.05) in G2 and G3 than in G1, being the highest in G3. Each of PCV, Hb and RBCs were higher (P<0.05) in G2 and G3 than in G1, being the highest in G3, while WBCs showed (P<0.05) an opposite trend (P<0.05). Each of Hb, RBCs and WBCs increased (P<0.05) one month after treatment, then Hb and RBC increased (P<0.05), while PCV and WBCs decreased (P<0.05) at the 4th collection month. Serum TP, AL and GLU increased (P<0.05) in G3 as compared to G1 and G2, while GL was not affected by treatment. By advancing collection month, concentration of TP, AL, GL and glucose showed gradual increase (P<0.05). Concentration of TG and TC reduced (P<0.05) in G2 and G3, while HDL increased (P<0.05) in G3 as compared to G1. However, LDL was not affected by treatment. Concentration of TG decreased (P<0.05), while HDL increased (P<0.05) by advancing collection month. Concentration of TC and LDL showed fluctuated trend of change at different collection months (P<0.05). Serum urea decreased (P<0.05) in G3, while uric acid, creatinine, AST and ALT decreased (P<0.05) in G2 and G3 as compared to G1. Urea and uric acid decreased (P<0.05) during one month before semen collection and at the 3rd collection month, respectively. However, Cr, AST and ALT decreased (P<0.05) by advancing collection month. Catalase, GSH and SOD increased (P<0.05) in G2 and G3, being the highest in G3. All antioxidant enzymes increased (P<0.05) by advancing collection month, being at higher rate for SOD, followed by GSH and the lowest for catalase during month pre-treatment. The current study can conclude that, *moringa oleifera* leaves could be used as feed additive to help farmers for sustainable development of breeding bulls. Results of this study recommended that daily adding 240 g *moringa oleifera* leaves per buffalo bull for one month pre-semen collection or at a level of 8% of concentrate feed mixture in diets of buffalo bulls can improve quality and production of semen without any adverse effects on health status under hot climatic conditions in Egypt.

**Keywords:** Buffalo, blood, semen, moringa leaves, physiological parameters, hematological parameters.

## INTRODUCTION

Buffaloes played an important role in livestock production by providing the milk, meat, leather and work draft force (Andrabi, 2009; Kumar *et al*., 2011). The domestic buffaloes are distinct species within the *bovidae* family, having optimum climatic conditions to growth and reproduction (Payne, 1990). In heat stress condition buffaloes respond to high temperature by elevating body temperature (Mullick, 1960). Climatic change represented in high temperature with humidity depresses animal productive and reproductive efficiency (Omran, 2008; West, 2003). Using fertile bulls with high semen quality leads to increasing conception and reducing the culling rate of buffalo cows. To increase the reproductive performance of buffalo cows, buffalo bulls used for natural mating or as semen donors for artificial insemination (AI) should have semen of good quality (Kastelic, 2013). Buffalo semen quality is influenced by different factors, including nutrition (Martin *et al*., 2010), breed (Lemma and Shemsu, 2015), age and season (Bhakat *et al*., 2011). Bull nutrition status affects sperm production by controlling gonadotropin secretion and sexual development. Spermatogenesis requires amino acids (arginine, methionine and cysteine) (Young *et al*., 2008; Wu *et al*., 2009), fatty acids (α-linoleic), vitamins (Vit. A, C, and E) and minerals (Zn and Se) (Cheah and Yang, 2011). In Bali bulls, Syarifuddina *et al*. (2017) found that, supplementation of Moringa *oleifera* leaves (MOL) increased plasma testosterone concentrations and sperm motility.

Bio-constituents such as saponin, alkaloid, flavonoid, ferulic acid, and chlorogenic acid in some herbs are responsible for enhancing spermatogenesis (Chauhan *et al*., 2014). In this respect, *Moringa oleifera* (MO) is one of the plants that contain all of these compounds and it is a good alternative for fodder crops, especially in the dry season when no fodder is available (Nouman *et al*., 2013). The MO is one of the *Moringaceae* family which belong to the genus called Moringa the most widely known and utilized specie grown worldwide in the tropics and

subtropics, native from the sub Himalayan region of North-West India, Pakistan, Bangladesh and Afghanistan, is also indigenous to other countries (Melo *et al*., 2013). It's used as feedstuff for large and small ruminants (Fayomi *et al*., 2014) and as feed additive for livestock (Fitri *et al*., 2015). Moreover, MO leaves seem to have strong antioxidant properties. Compounds such as polyphenols, tannins, anthocyanin, glycosides and thiocarbamates in MO leaves can inhibit oxidases by removed free radicals and activate antioxidant enzymes (Luqman *et al*., 2012), and can prevent lens of rats from morphological changes and oxidative damage by enhancing the activities of antioxidant enzymes (Sreelatha and Padma, 2009).

In Egypt, buffaloes exposed to heat stress for long duration try to acclimatize in the adverse condition, the time required for acclimation found to be within 24-48 h (Omran, 2008). Leaves of MO is a potential inexpensive protein source for livestock feed (Sarwatt *et al*., 2004), and it had strong effect on physiological parameters including rectal temperature, respiratory and pulse rate (Anwar *et al*., 2007 and Babeker and Abdalbagi, 2015). In addition, feeding the MO leaves has pronounced effects on reducing lipid profile in rats (Lewis and Rader, 2005) and human (Seriki *et al*., 2015), and on all hematological parameters of West African Dwarf rams (Akinyemi *et al*., 2010) and in yearling yankasa rams (Fayomi *et al*., 2014).

Recently, the usage of MO leaf extract as oral administration (El-Harairy *et al*., 2016; Khalifa *et al*., 2016) for improving semen quality or effects of graded levels of MO leaf meal on the testicular morphometry and sperm quality were studied on rabbit bucks. However, no available information on the effect of MO leaves as a dietary addition on reproductive performance of buffalo bulls kept under hot climatic conditions in Egypt. Therefore, the current study aimed to evaluate dietary supplementation of Moringa oleifera leaves on semen quality, oxidative stress, thermal regulation and health status of heat stressed buffalo bulls.

## MATERIALS AND METHODS

The present study was conducted at Animal Production Research Station, El-Gemmezah, belonging to Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture, Egypt, during the period from April to October 2015.

**Animals:**

Eighteen sexually mature Egyptian buffalo bulls with good healthy appearance (3 years old and 400-420 kg LBW) were divided into three groups, 6 animals in each. Bulls in the first group were fed daily on ration composed of 3 kg concentrate fed mixture (CFM), 4 kg berseem hay (BH) and 4 kg rice straw (RS) according to NRC, (1988) requirements without any additive as control. Bulls in the second and third groups were fed the same control diet supplemented with *Moringa oleifera* leaves (MOL) at levels of 4 and 8% of CFM, respectively. Feeding period of the experimental diets was for one month pre-semen collection and during semen collection period (4 months). The CFM was composed of 65% un- corticated cotton seed cake, 9% wheat bran, 20% rice polish, 3% molasses, 2% limestone and 1% sodium chloride. Bulls were given individual feeds twice daily at 8.00 a.m. and 3.0 p.m., while clean water was available all day time. Animals were housed individually under semi-open sheds.

**Semen sampling and evaluation:**

Semen was collected twice weekly using an artificial vagina (IMV, France) at 7-8 a.m. before morning feeding using teaser bull. Immediately after ejaculation, semen was kept at 35-37◦C in water bath and taken immediately to the laboratory. Semen collection period lasted for 4 months

Ejaculate semen volume was measured and evaluated for percentages of individual motility (Amman and Hammerstedt 1980), livability (Hackett and Macpherson 1965), abnormality (Blom, 1983) and acrosomal status (Yanagimachi, 1982) of spermatozoa. However, the response of bull spermatozoa to HOS-test was assessed in term of percentage of curled spermatozoa at 50 mOsm/l for 30 min according to El-Sherbieny (2004).

Seminal plasma was obtained by centrifugation 2 ml of semen at 3000 rpm for 25 minutes at room temperature according to Khan *et al*. (2015), the supernatant stored at deep freezer at -70ºC until further analyses.

**Climatic condition:**

Environmental ambient temperature (AT, °C) was estimated as the average of highest and lowest air temperature among day, relative humidity (RH%) were recorded and temperature-humidity index (THI) was estimated (Table 1) during treatment period according to Thom (1959) using the following formula:

**THI = (0.8 x AT °C) + [(RH/100) x (AT °C - 14.4)] + 46.4**

THI < 72 = absence of heat stress, 72 to < 74 = moderate heat stress, 74 to < 78 = severe heat stress and more than 78 = very severe heat stress.

**Table 1. Ambient temperature (AT,°C), relative humidity (RH, %) and temperature-humidity index (THI) during the experimental period.**

| Experimental period | AT (°C) | RH (%) | THI |
|---|---|---|---|
| 0 time (Pre-treatment and collection) | 24.13±0.85 | 33.00±1.58 | 68.88±0.86 |
| 1st collection month | 29.63±1.46 | 51.03±0.71 | 77.85±1.83 |
| 2nd collection month | 31.38±1.20 | 47.40±1.93 | 79.55±1.53 |
| 3rd collection month | 32.50±0.41 | 49.73±2.64 | 81.39±0.64 |
| 4th collection month | 30.75±0.43 | 34.90±0.42 | 76.71±0.53 |

**Physiological response:**

Rectal temperature (RT, °C) skin temperature (ST, °C), respiration rate (RR, r/min) and heart pulse rate (PR, p/min) were recorded once a weak through the experimental period using digital thermometer and stop watch.

**Blood sampling:**

Blood samples were individually collected from the jugular vein in two test tubes (with and without coagulant) for each animal. Blood samples were taken pre-treatment and during 1st, 2nd, 3rd and 4th months of semen collection. In the 1st portion (without coagulant), blood samples were left to clot for about 2-3 h, then serum was carefully obtained by centrifugation at 3000 rpm for 20 minutes and stored at -20°C until performing chemical analyses. In the 2nd portion (with coagulant), hematological parameters including

hemoglobin (Hb) and packed cell volume (PCV%) were directly determined using Mission® Plus kit (REF C132-3031, USA) (Henry, 2001), while red (RBCs) and white (WBCs) blood cells were counted using hemocytometer.

**Analytical procedures:**

Blood serum were analyzed for concentration of total proteins (Henry, 1964), albumin (Doumas *et al*., 1971), glucose (Trinder, 1969), triglycerides (Mc Gowan *et al*., 1983), total cholesterol (Richmond, 1973), high-density lipoprotein (HDL), low-density lipoprotein (LDL) (Friedewald *et al*., 1972), urea (Bull *et al*., 1991), uric acid (Caraway, 1963) and creatinine (Bartles *et al*., 1972), using commercial kits (Nanjing Jiancheng Biochemical Re-agent Co., Nanjing, China). Globulin concentration was obtained by subtracting the values of serum albumin from the corresponding values of total proteins,

Enzyme activity of asprtate (AST) and alanine (ALT) transaminases (Reitman and Frankel, 1957), superoxide dismutase, SOD (Madesh and Balasubramanian, 1998), catalase (Bergmeyer, 1983), and glutathione (Prins, and Loos, 1969) were determined in blood serum.

Concentration of testosterone in blood serum was estimated by radioimmuno assay according to the procedure described by Ekins (1984).

Also, seminal plasma were analyzed for triglycerides, total cholesterol, activity of AST, ALT, SOD, Catalase and glutathione with the same method of blood serum analysis.

**Statistical analysis:**

Data were processed with the SPSS analysis program (SPSS, 2013) as a factorial design to study the effect of experimental group (1….3), sampling months (1…..5 for all data; 1….4 for sperm characteristics ) and their interaction. The detected significant differences were performed at P<0.05 by Duncan Multiple Range Test (Duncan, 1955). Values were set as mean ± standard error for each month of sampling time.

## RESULTS AND DISCUSSION

**Physiological responses:**

Overall mean of physiological response of buffalo bulls in different experimental groups (Table 2), in terms of rectal (RT) and skin (ST) temperature degrees, respiration rat (RR) and pulse rate (PR), significantly (P<0.05) decreased in G3 than in G2 and G1, reflecting the highest physiological response of bulls fed diet supplemented with Moringa oleifera leaves (MOL) at a high versus low level (8 vs. 4% of CFM).

Overall mean of physiological response of buffalo bulls at different experimental months showed significantly (P<0.05) gradual increase up to 3rd collection month (maximum values of RT, ST, RR and PR), then decreased at the 4th collection month. It is of interest to note that this trend of change in physiological response was associated with change in THI values, being the highest at the 3rd collection month (81.39), representing very severe heat stress for THI values ≥78. Effect of interaction between treatment and collection month on all physiological response parameters was not significant (Table 2).

The domestic buffaloes have optimum climatic conditions to growth and reproduction as follows, 13–18°C air ambient temperature in combined with 55–65% relative humidity and medium level of sunshine (Payne, 1990). The presented data are within the normal range of Egyptian buffalo physiological parameters under high air temperature (Omran *et al*., 2013). Previous studies suggested that, when buffalo exposure to direct solar radiation, body temperature rise (Mullick, 1960). In accordance with the present results, Babeker and Abdalbagi (2015) reported similar physiological response of goats fed MOL diets under hot environmental condition.

**Table 2. Mean and standard error of physiological response of Egyptian buffalo bulls as affected by dietary MOL supplementation, collection month and their interaction.**

| Item | Rectal temperature (°C) | Skin temperature (°C) | Respiration rate (time/min) | Pulse rate (pulse/min) |
|---|---|---|---|---|
| | | Effect of treatment (T): | | |
| G1 (control) | 39.00±0.18$^{a}$ | 36.83±0.12$^{a}$ | 64.30±2.11$^{a}$ | 47.30±1.36$^{a}$ |
| G2 (120 g/d/h MOL) | 38.76±0.17$^{ab}$ | 36.48±0.10$^{b}$ | 61.47±1.92$^{a}$ | 45.23±1.12$^{a}$ |
| G3 (240 g/d/h MOL) | 38.29±0.18$^{b}$ | 35.92±0.11$^{c}$ | 57.00±1.90$^{b}$ | 42.33±0.94$^{b}$ |
| | | Effect of collection month (M): | | |
| 0 time | 38.31±0.20$^{b}$ | 36.21±0.10$^{b}$ | 49.89±1.84$^{d}$ | 41.17±1.30$^{c}$ |
| 1$^{st}$ collection month | 38.55±0.22$^{b}$ | 36.27±0.20$^{b}$ | 57.39±1.90$^{c}$ | 42.72±1.21$^{c}$ |
| 2$^{nd}$ collection month | 38.82±0.23$^{ab}$ | 36.48±0.15$^{ab}$ | 65.33±2.25$^{b}$ | 46.06±1.29$^{b}$ |
| 3$^{rd}$ collection month | 39.33±0.24$^{a}$ | 36.80±0.17$^{a}$ | 71.33±1.99$^{a}$ | 52.44±1.34$^{a}$ |
| 4$^{th}$ collection moth | 38.42±0.23$^{b}$ | 36.30±0.16$^{b}$ | 60.67±2.15$^{bc}$ | 42.39±1.06$^{c}$ |
| | | Effect of interaction (T x M): | | |
| P-value | 0.899 | 0.070 | 0.958 | 0.229 |

**Means denoted within the same column for each factor with different superscripts are significantly different at P<0.05. 0 time: pre-treatment and semen collection.**

**Reproductive parameters:**

***Sperm characteristics:***

Overall mean of sperm characteristics in semen of buffalo bulls in different experimental groups (Table 3), including percentage of individual motility (IM), livability (SL), abnormality (SA), curled tail (CT) and damage acrosome (DA) of spermatozoa, were significantly (P<0.05) improved in G2 and G3 as compared to G1, being significantly (P<0.05) the best in G3, reflecting impact of feeding bulls on diet supplemented with MOL at 8% of CFM.

Overall mean of sperm characteristics in semen of buffalo bulls showed significantly (P<0.05) gradual improvement by advancing collection month, being the best at the 4th collection month. It is worthy noting that the effect of interaction between treatment and collection month on all sperm characteristics studied was significant at P<0.001 (Table 2). This effect reflected marked increase in percentages of sperm IM, SL and CT versus pronounced reduction in percentage of SA and DA in G2 and G3 by advancing collection month as compared to G1 (control) as illustrated in figure 1a, b, c, d and e, respectively. Such results

indicated beneficial effects of dietary supplementation with MOL on sperm function to have ability of sperm movement within the female reproductive tract beside high fertilizing ability. In this respect, Perumal *et al*. (2014) recorded a positive correlation between sperm motility percentage and fertility.

In accordance with the present results, using MOL significantly increased percentage of motility, livability and membrane integrity of rabbit bucks, as an extract (Khalifa *et al*., 2016) or as a meal (Oyeyemi *et al*., 2008). In this respect, Dacheux *et al*. (2003) found that epididymis is known to play a major role in the final development of motility, fertilizing ability and sperm storage. These results may suggest the pronounced effects of MOL as antioxidant on improving most sperm characteristics, including motility, livability and abnormality of spermatozoa and may be attributed to the prevention of excessive generation of free radicals produced by sperm by means of the antioxidant properties of MOL. Also, Purdy *et al*. (2004) demonstrated that flavonoids caused an increase sperm motility. Moreover, Eid *et al*. (2006) found that a higher antioxidant intake was associated with greater sperm numbers and motility. These results supported the obtained results concerning improvement in sperm motility in association with reducing sperm abnormality without pronounced effect on sperm livability as affected by MOL treatment.

**Table 3. Mean and standard error of sperm characteristics in buffalo semen as affected by dietary MOL supplementation, collection month and their interaction.**

| Item | Sperm characteristics (%) | | | | |
|---|---|---|---|---|---|
| | Individual motility | Livability | Abnormality | Curled tail | Damage acrosome |
| Effect of treatment (T): | | | | | |
| G1 (control) | 65.52±0.55$^{c}$ | 63.64±0.36$^{c}$ | 29.80±0.27$^{a}$ | 60.89±0.43$^{c}$ | 29.33±0.29$^{a}$ |
| G2 (120 g/d/h MOL) | 71.82±0.69$^{b}$ | 69.22±0.65$^{b}$ | 22.80±0.51$^{b}$ | 66.67±0.69$^{b}$ | 24.66±0.32$^{b}$ |
| G3 (240 g/d/h MOL) | 74.53±0.80$^{a}$ | 72.16±0.78$^{a}$ | 19.95±0.68$^{c}$ | 69.78±0.95$^{a}$ | 21.04±0.48$^{c}$ |
| Effect of collection month (M): | | | | | |
| 1$^{st}$ collection month | 66.94±0.60$^{d}$ | 62.92±0.35$^{d}$ | 28.78±0.23$^{a}$ | 60.25±0.49$^{d}$ | 27.17±0.24$^{a}$ |
| 2$^{nd}$ collection month | 68.89±0.83$^{c}$ | 66.58±0.58$^{c}$ | 24.60±0.49$^{b}$ | 62.93±0.62$^{c}$ | 25.46±0.45$^{b}$ |
| 3$^{rd}$ collection month | 72.22±0.87$^{b}$ | 69.35±0.73$^{b}$ | 23.24±0.69$^{c}$ | 68.49±0.74$^{b}$ | 23.97±0.59$^{c}$ |
| 4$^{th}$ collection month | 74.44±1.03$^{a}$ | 74.50±0.88$^{a}$ | 20.13±1.04$^{d}$ | 71.43±1.13$^{a}$ | 23.44±0.83$^{c}$ |
| Effect of interaction (T x M): | | | | | |
| P value | 0.000*** | 0.000*** | 0.000*** | 0.000*** | 0.000*** |

**Means denoted within the same column for each effect with different superscripts are significantly different at P<0.05.**

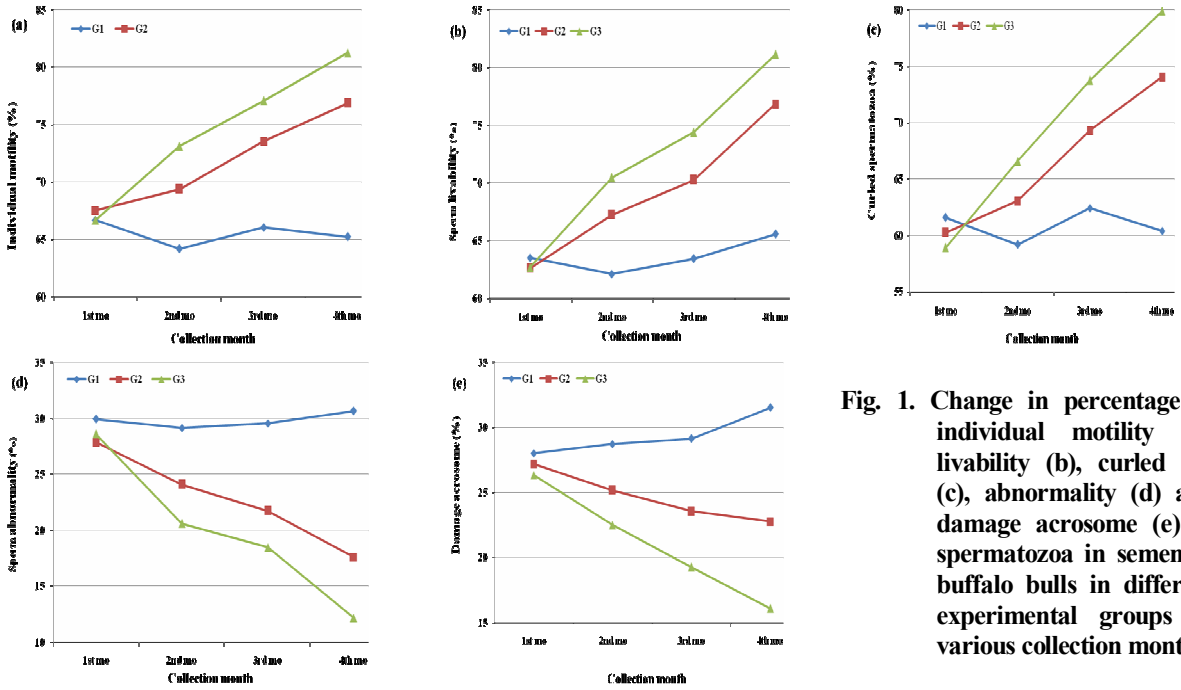


**Fig. 1. Change in percentage of individual motility (a), livability (b), curled tail (c), abnormality (d) and damage acrosome (e) of spermatozoa in semen of buffalo bulls in different experimental groups at various collection months.**

**Seminal plasma characteristics:**

Results in Table 4 cleared significant (P<0.05) decrease in overall mean of total cholesterol (TC) and triglycerides (TG) concentrations in seminal plasma of G3 as compared to G1 and G2. However, overall mean of AST and ALT activity significantly (P<0.05) decreased, while overall mean of catalase, glutathione (GSH) and SOD activities significantly (P<0.05) increased in seminal plasma of G2 and G3 as compared to G1. This means that dietary MOL supplementation had marked effect on decreasing concentration of TC and TG as well as increasing activity of AST, ALT, catalase, GSH and SOD in seminal plasma, particularly MOL at a level of 8% of CFM.

As affected by collection month, overall mean of TC concentration and activity of ALT, catalase, GSH and SOD in seminal plasma significantly (P<0.05) increased, while TG concentration significantly (P<0.05) decreased by advancing collection month. However, AST activity showed insignificant changes at different collection months (Table 4).

Effect of interaction between MOL treatment and collection month was significant on activity of AST, ALT, GSH (P<0.001) and SOD (P<0.05, Table 4). This effect was reflected in inconsistent trend of change in

AST and ALT activity at different collection weeks (Fig. 2 a & b), while GSH and SOD activities were the highest in G3, followed by G2 and the lowest in G1 at most collection months (Fig. c & d).

**Table 4. Mean and standard error of some biochemicals and antioxidant enzymes in seminal plasma of Egyptian buffalo bulls as affected by dietary MOL supplementation, collection month and their interaction.**

| Item | Some biochemicals and enzyme activity in seminal plasma | | | | | | |
|---|---|---|---|---|---|---|---|
| | Cholesterol (mg/dl) | Triglycerides (mg/dl) | AST (U/L) | ALT (U/L) | Catalase (mg/dl) | GSH (mg/dl) | SOD (mg/dl) |
| Effect of treatment (T): | | | | | | | |
| G1 (control) | 101.55±2.38$^{a}$ | 43.32±1.09$^{a}$ | 0.219±0.012$^{a}$ | 0.171±0.008$^{a}$ | 6.47±0.34$^{b}$ | 10.55±0.38$^{c}$ | 14.21±0.73$^{b}$ |
| G2 (120 g/d/h MOL) | 94.74±3.32$^{a}$ | 42.66±1.12$^{a}$ | 0.185±0.005$^{b}$ | 0.151±0.005$^{b}$ | 8.22±0.55$^{a}$ | 13.14±0.47$^{b}$ | 16.48±0.85$^{a}$ |
| G3 (240 g/d/h MOL) | 86.46±3.13$^{b}$ | 37.16±1.15$^{b}$ | 0.163±0.006$^{c}$ | 0.157±0.006$^{b}$ | 8.60±0.62$^{a}$ | 14.10±0.64$^{a}$ | 17.00±0.84$^{a}$ |
| Effect of collection month (M): | | | | | | | |
| 0 time | 95.75±1.40$^{ab}$ | 46.16±0.50$^{a}$ | 0.192±0.005 | 0.153±0.007$^{b}$ | 5.64±0.31$^{c}$ | 8.99±0.27$^{d}$ | 10.21±0.52$^{c}$ |
| 1$^{st}$ collection month | 88.78±4.91$^{b}$ | 46.72±1.38$^{a}$ | 0.192±0.003 | 0.157±0.006$^{b}$ | 6.06±0.52$^{c}$ | 11.42±0.59$^{c}$ | 14.28±0.95$^{d}$ |
| 2$^{nd}$ collection month | 89.30±4.18$^{b}$ | 38.79±1.70$^{b}$ | 0.190±0.022 | 0.146±0.009$^{b}$ | 7.53±0.41$^{b}$ | 13.23±0.63$^{b}$ | 16.35±0.54$^{c}$ |
| 3$^{rd}$ collection month | 94.52±4.69$^{ab}$ | 34.96±0.72$^{c}$ | 0.181±0.009 | 0.152±0.005$^{b}$ | 8.04±0.25$^{b}$ | 14.03±0.60$^{b}$ | 18.21±0.86$^{b}$ |
| 4$^{th}$ collection month | 102.89±3.64$^{a}$ | 38.64±1.09$^{b}$ | 0.189±0.008 | 0.189±0.011$^{a}$ | 11.55±0.81$^{a}$ | 15.33±0.52$^{a}$ | 20.42±0.53$^{a}$ |
| Effect of interaction (T x M): | | | | | | | |
| P-value | 0.238 | 0.127 | 0.000*** | 0.000*** | 0.080 | 0.001*** | 0.020** |

**Means denoted within the same column with different superscripts are significantly different at P<0.05. 0 time: pre-treatment and semen collection. AST: Asprtate amino transaminase. ALT: Alanine amino transaminase. GSH:Glutathione. SOD: Superoxide dismutase.**

The biochemical components in seminal plasma play a pivotal role in providing substrate energy forming essential link in the energy generating cycles in sperm metabolism in the process of fertilization and in the maintenance of constant osmotic pressure during semen preservation (Dhami and Kodagali, 1987). Estimation of these biochemicals in ejaculated semen or directly in the glands can be used as an index of accessory glands function (White, 1976). The observed reduction in TC concentration in seminal plasma of bulls in G3 may be due to marked decrease in TC level in blood as affected by MOL supplementation. In this respect, Ghasi *et al.* (1999) noted that extracted liquid of MOL was an effective hypo-cholesterolemic agent. In their study using Wister rat, when given a low level of 1 mg/g, with diet higher in fat daily more than 30 days (experimental period), cholesterol was decrease in serum, kidney and liver. Furthermore, MO leaves significantly decreased concentrations of TC and TG in blood serum in hyper-cholesterolaemic Wistar rats. Importantly, it was demonstrated by Chumark *et al.* (2008) that the extract MOL could decrease TC and TG concentrations in rabbits at scales similar to folks of simvastatin.

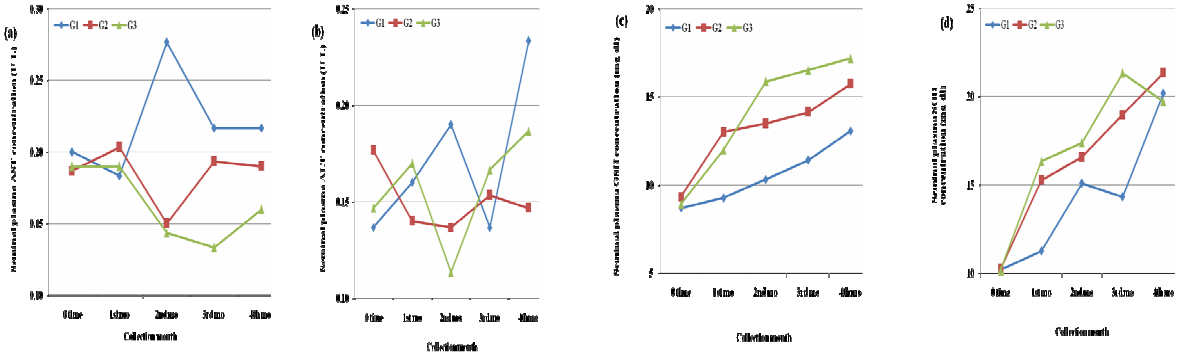


**Fig. 2. Change in activity of AST (a), ALT (b), GSH (c) and SOD (d) in seminal plasma of buffalo bulls in different experimental groups at various collection months.**

On the other hand, the recent results of Nuhu (2010) showed that MOL meal had no effect significantly (P > 0.05) on blood cholesterol concentration. Ahemen *et al.* (2013) reported that deit of MO did not affected significantly (p>0.05) on concentration of TC in serum.

Concentration of AST and ALT enzymes in semen is a good indicator of semen quality. The release of enzymes has been shown to be associated with sperm cell injury Activity of AST and ALT in seminal plasma is mostly contributed by sperm cells. Generally, activity of ASTwas almost higher than ALT as estimated by several authors in seminal plasma (Rasul *et al.*, 1999). Level of transaminases in seminal is an indication of sperm death and of sperm membrane damage Daader *et al.* (1993) reported that level of transaminases in seminal is an indication of sperm death and of sperm membrane damage. In accordance with the present results, Eshak and Osman (2013) observed that MOL aqueous extract had a therapeutic action through enhancing of liver enzyme activities (AST, ALT and ALK) in irradiated rats by gamma irradiation. In the present study, seminal plasma of bulls in G3 showed the lowest AST and ALT activity with the best semen quality. Similarly, Daader *et al.* (1993) reported that percentage of live sperm in semen was correlated negatively with AST and ALT activity. Also, Abdel-Gawad *et al.* (2000) found increases in the concentration of AST and ALT enzymes in goat seminal plasma (probably from prostatic origin) and this was associated with high percentage of dead and abnormal spermatozoa.

In harmony with increasing activity of antioxidant enzymes in seminal plasma (catalase, GSH and SOD) may be attributed to increasing total antioxidant activity and decreasing lipid peroxidas in blood of rabbit bucks treated with MO extract (El-Harairy *et al.*, 2016). Also, Afolabi *et*

*al*. (2013) reported that SOD activity increased in group treated with MOL extract as compared to control, which may due to the high content of MOL from flavonoids as an antioxidant component (Asma *et al*., 2005).

**Testosterone concentration:**

Serum testosterone concentrations of bulls were significantly (P<0.05) higher in bulls fed diet supplemented with MOL than in control bulls, being the highest in G3. It is of interest to observe that the recorded increase in testosterone in MOL groups is in relation to pronounced improvement in sperm characteristics, particularly in G3. These results are in accordance with the study of Prabsattroo *et al*. (2015) and Dafaalla *et al*. (2016) in rats. Chauhan *et al*. (2014) reported that MOL contain bio-constituents which are responsible for enhancing sexual activity and spermatogenesis include saponin, alkaloid, flavonoid, ferulic acid, and chlorogenic acid. Ghasi *et al*. (1994) found that MOL are containing β-sitosterol which preserve and enhance the process of spermatogenesis in mice. Plant extracts might have a role in testosterone secretion allowing better availability of hormone to gonads (Amini and Kamkar, 2005).One of MOL supplementation mode of action may be by increasing Leydig cells (Prabsattroo *et al*., 2015) and FSH and LH levels (Dafaalla *et al*., 2016), then Leydig cells performing the synthesis of testosterone performed in the testes depends on the adequacy of Zn in the diet (Roy *et al*., 2013). The MOL had suitable quantity of Zn and β–sitosterol which found to preserve the process of spermatogenesis in mice (Ghasi *et al*., 1994).

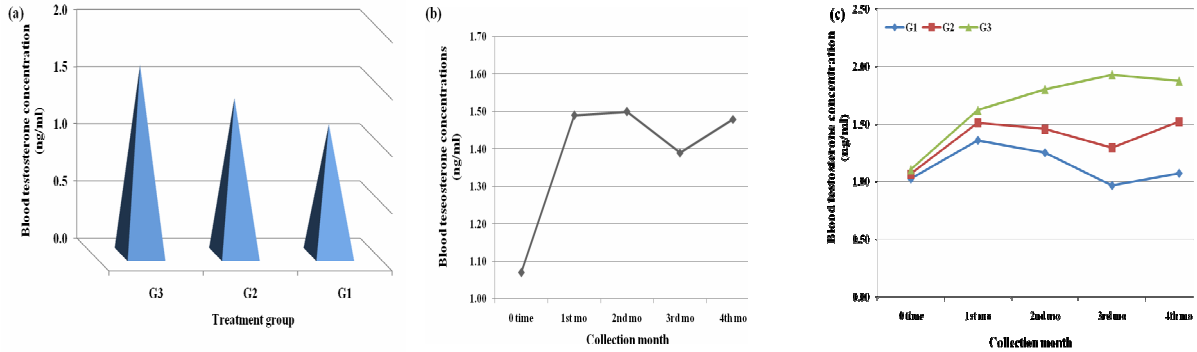


**Fig. 3. Testosterone concentration in blood plasma of buffalo bulls as affected by MOL treatment (a), collection month (b), and their interaction (c).**

**Blood parameters:**

**Hematological parameters:**

Hematological parameters including packed cell volume ( PCV), hemoglobin concentration (Hb) and count of red blood cells (RBCs) were significantly (P<0.05) higher in G2 and G3 than in G1, being the highest in G3. However, count of white blood cells (WBCs) showed significantly (P<0.05) an opposite trend (Table 5).

Results also showed significant (P<0.05) increase Hb, RBCs and WBCs one month after treatment, then Hb and RBC significantly (P<0.05) increased, while PCV and WBCs significantly (P<0.05) decreased at the 4th collection month. However, the effect of interaction between MOL treatment and collection month was significant only on PCV and WBCs (Table 5). The significant interaction on PCV was due to similar trend of reduction up to 2nd month of collection, followed by increase to 3rd month and other reduction at 4th month in G2 and G3 versus continued reduction in PCV in G1 (Fig. 4a). The significant interaction on WBCs was reflected in inconsistent trend of change in WBCs in all groups by advancing collection month (Fig. 4b). All hematological parameters obtained in the current study are within normal ranges of buffalo bulls (Omran *et al*., 2013). Similar results were obtained by Babeker and Abdalbagi (2015) on Sudan Nubian goats fed on MOL. In agreement with Omran (2008), both PCV and Hb were significantly (P<0.05) decreased as affected by high air temperature, reaching the lowest values at 3rd collection week with very sever heat stress condition during this month. Genrally, results of hematological parameters are a reflection for animal response to external environment (Isikwenu *et al*., 2012). Also, Akinyemi *et al*. (2010) showed that all hematological parameters of West African Dwarf rams were best with dietary inclusion of MO. Moreover, Fayomi *et al*. (2014) found that dietary inclusion of MO had positive effect on blood hematological profiles of yearling rams,.

**Table 5. Mean and standard error of hematological parameters of Egyptian buffalo bulls as affected by dietary MOL supplementation, collection month and their interaction.**

| Item | Packed cell volume (%) | Hemoglobin (mg/dl) | Red blood cells ($x10^6/mm^3$) | White blood cells ($x10^3/mm^3$) |
|---|---|---|---|---|
| Effect of treatment (T): | | | | |
| G1 (control) | $31.39\pm0.35^{c}$ | $7.93\pm0.17^{c}$ | $7.05\pm0.13^{c}$ | $7.80\pm0.15^{a}$ |
| G2 (120 g/d/h MOL) | $33.69\pm0.19^{b}$ | $9.15\pm0.19^{b}$ | $7.49\pm0.11^{b}$ | $7.41\pm0.12^{b}$ |
| G3 (240 g/d/h MOL) | $34.84\pm0.15^{a}$ | $10.05\pm0.20^{a}$ | $7.94\pm0.15^{a}$ | $7.15\pm0.11^{c}$ |
| Effect of collection month (M): | | | | |
| 0 time | $34.51\pm0.14^{a}$ | $8.34\pm0.22^{c}$ | $6.76\pm0.20^{c}$ | $6.63\pm0.14^{c}$ |
| 1st collection month | $33.54\pm0.31^{b}$ | $9.07\pm0.27^{b}$ | $7.39\pm0.08^{b}$ | $8.09\pm0.16^{a}$ |
| 2nd collection month | $32.42\pm0.42^{d}$ | $8.94\pm0.37^{b}$ | $7.36\pm0.13^{b}$ | $7.42\pm0.12^{b}$ |
| 3rd collection month | $33.24\pm0.59^{bc}$ | $8.68\pm0.18^{bc}$ | $8.02\pm0.12^{a}$ | $7.66\pm0.10^{b}$ |
| 4th collection month | $32.81\pm0.57^{cd}$ | $10.18\pm0.32^{a}$ | $7.93\pm0.21^{a}$ | $7.44\pm0.15^{b}$ |
| Effect of interaction (T x M): | | | | |
| P-value | 0.000*** | 0.125 | 0.107 | 0.037** |

**Means denoted within the same column with different superscripts are significantly different at P<0.05. 0 time: Pre-treatment and semen collection**

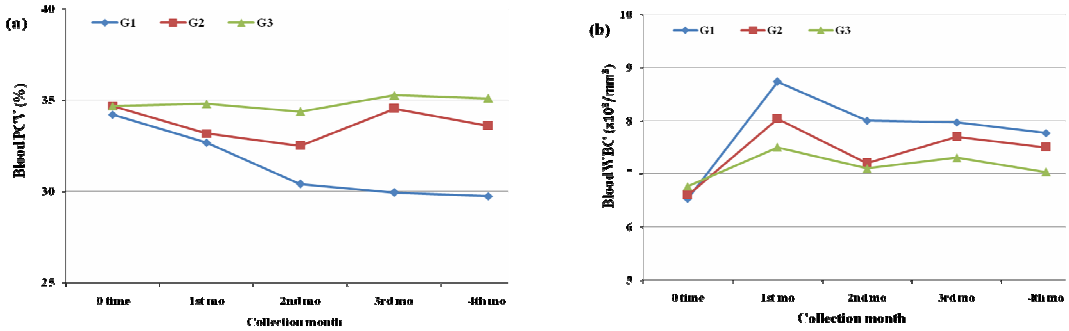


**Fig. 4. Change in PCV% (a) and Hb concentration (b) in blood of buffalo bulls of different experimental groups at various collection months.**

**Protein metabolism:**

Bulls treated with MOL in G3 showed significant (P<0.05) increase in concentration of total proteins (TP), albumin (AL) and glucose in blood serum as compared to G1 and G2, while globulin (GL) concentration was not affected by treatment. By advancing collection month, concentration of TP, AL, GL and glucose showed significantly (P<0.05) gradual increase (Table 6).

**Table 6. Mean and standard error of some serum biochemicals of Egyptian buffalo bulls as affected by dietary MOL supplementation, collection month and their interaction.**

| Item | Total proteins (g/dl) | Albumin (g/dl) | Globulin (g/dl) | Glucose (mg/dl) |
|---|---|---|---|---|
| Effect of treatment (T): | | | | |
| G1 (control) | 6.93±0.21$^{b}$ | 3.22±0.13$^{b}$ | 3.71±0.16 | 60.84±1.21$^{b}$ |
| G2 (120 g/d/h MOL) | 7.25±0.19$^{b}$ | 3.17±0.12$^{b}$ | 4.08±0.14 | 62.64±1.19$^{b}$ |
| G3 (240 g/d/h MOL) | 8.74±0.29$^{a}$ | 4.73±0.26$^{a}$ | 4.01±0.15 | 65.89±1.54$^{a}$ |
| Effect of collection month(M): | | | | |
| 0 time | 6.53±0.24$^{c}$ | 2.47±0.03$^{e}$ | 4.06±0.25$^{ab}$ | 59.89±1.35$^{c}$ |
| 1$^{st}$ collection month | 6.84±0.35$^{bc}$ | 3.10±0.22$^{d}$ | 3.74±0.26$^{ab}$ | 64.43±1.31$^{ab}$ |
| 2$^{nd}$ collection month | 7.30±0.22$^{b}$ | 3.75±0.28$^{c}$ | 3.56±0.16$^{b}$ | 61.25±2.25$^{bc}$ |
| 3$^{rd}$ collection month | 8.67±0.32$^{a}$ | 4.47±0.24$^{b}$ | 4.20±0.15$^{a}$ | 67.01±1.85$^{a}$ |
| 4$^{th}$ collection month | 8.85±0.22$^{a}$ | 4.73±0.20$^{a}$ | 4.11±0.06$^{a}$ | 63.03±1.56$^{abc}$ |
| Effect of interaction (T x M): | | | | |
| P-value | 0.002** | 0.000*** | 0.036* | 0.000*** |

**Means denoted within the same column with different superscripts are significantly different at P<0.05. 0 time : Pre-treatment and semen collection.**

Effect of interaction between MOL treatment and collection month was significant on all parameter studied (Table 6). Consequently, TP (Fig. 5 a) and AL (Fig. 5 b) concentrations showed nearly similar trend of increase by advancing collection month, being higher in G3 than in G1 and G2. However, GL (Fig. 5 c) and glucose (Fig. 5 c) showed inconsistent trend of change in all groups at different collection months. This results may indicated that increasing TP concentration was mainly related to increasing AL rather than GL concentration as affected by MOL treatment.

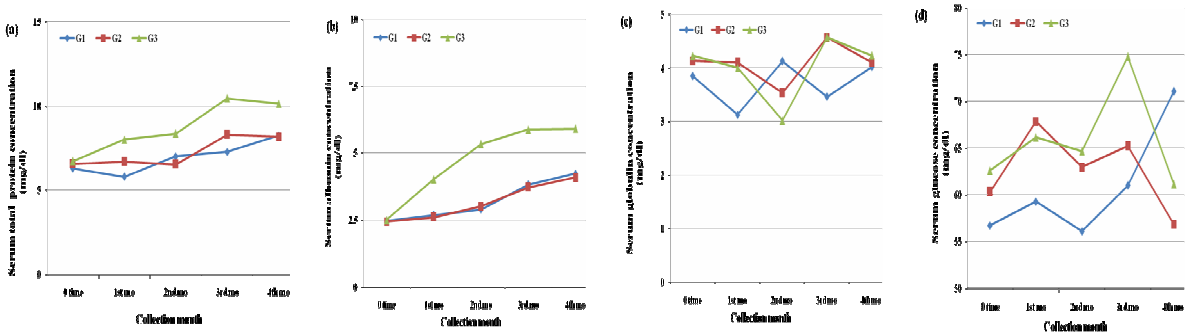


**Fig. 5. Change in concentration of total proteins (a), albumin (b), globulin (c) and glucose (d) in blood serum of buffalo bulls of different experimental groups at various collection months.**

According to Eggum (1970), blood total proteins depend on the quantity and quality of dietary protein. The superior concentration of total proteins in G3 may be refer to MOL chemical composition which may be increase rumen undegradable protein utilization (Garg *et al*., 1992) and improve synthesis of microbial protein in the rumen (Soliva *et al*., 2005). Also, feeding ruminants on MO can help in carbohydrates absorption and increasing metabolizable energy (Khalel *et al*., 2014). In this respect, Annison *et al*. (2002) found a linear relationship between glucose entry rate and metabolizable energy intake. However, Ahemen *et al*. (2013) reported insignificant influence of diet containing MOL on glucose in rabbit serum.

**Lipid profile:**

Lipid profile in blood serum was affected by MOL treatment, in terms of significant (P<0.05) reduction in concentration of triglycerides (TG) and cholesterol (TC) in G2 and G3, and significant (P<0.05) increase in HDL concentration in G3 only as compared to G1. However, LDL concentration was not affected by treatment (Table 7).

Concentration of TG significantly (P<0.05) decreased, while HDL concentration significantly (P<0.05) increased by advancing collection month. However, concentration of both TC and LDL showed significantly (P<0.05) fluctuated trend of change at different collection months (Table 7).

Interestingly to note that, the effect of interaction between treatment and collection month was significant on all lipid profile parameters, reflecting different trends of change in G1, G2 and G3 in concentration of TG (Fig. 6 a), TC (Fig. 6 b), LDL (Fig. 6 c) and HDL (Fig. 6 d).

**Table 7. Mean and standard error of lipid profiles in blood serum of Egyptian buffalo bulls as affected by MOL supplementation, collection month and their interaction.**

| Item | Triglycerides (mg/dl) | Total cholesterol (mg/dl) | LDL (mg/dl) | HDL (mg/dl) |
|---|---|---|---|---|
| | | Effect of treatment (T): | | |
| G1 (control) | 64.70±1.84$^{a}$ | 176.54±6.27$^{a}$ | 64.50±7.92 | 89.84±2.69$^{b}$ |
| G2 (120 g/d/h MOL) | 59.56±2.06$^{b}$ | 156.18±5.52$^{b}$ | 50.24±4.67 | 92.54±2.30$^{ab}$ |
| G3 (240 g/d/h MOL) | 52.18±2.39$^{c}$ | 148.39±4.95$^{b}$ | 55.98±4.61 | 94.74±3.19$^{a}$ |
| | | Effect of collection month (M): | | |
| 0 time | 69.14±1.15$^{a}$ | 157.70±3.10$^{ab}$ | 62.67±6.06$^{ab}$ | 85.49±1.96$^{c}$ |
| 1$^{st}$ collection month | 66.91±2.19$^{a}$ | 165.95±7.32$^{a}$ | 76.70±7.21$^{a}$ | 73.13±1.39$^{d}$ |
| 2$^{nd}$ collection month | 63.62±1.41$^{b}$ | 143.73±10.45$^{b}$ | 47.19±9.60$^{bc}$ | 92.00±2.92$^{b}$ |
| 3$^{rd}$ collection month | 51.75±2.43$^{c}$ | 163.62±5.27$^{a}$ | 39.19±4.12$^{c}$ | 104.27±2.05$^{a}$ |
| 4$^{th}$ collection month | 42.64±1.26$^{d}$ | 170.86±9.15$^{a}$ | 58.80±8.00$^{ab}$ | 106.97±1.30$^{a}$ |
| | | Effect of interaction (T x M): | | |
| P-value | 0.001*** | 0.002** | 0.002** | 0.000*** |

**Means denoted within the same column with different superscripts are significantly different at P<0.05.**
**LDL: Low density lipoprotein HDL: High density lipoprotein 0 time : Pre-treatment and semen collection**

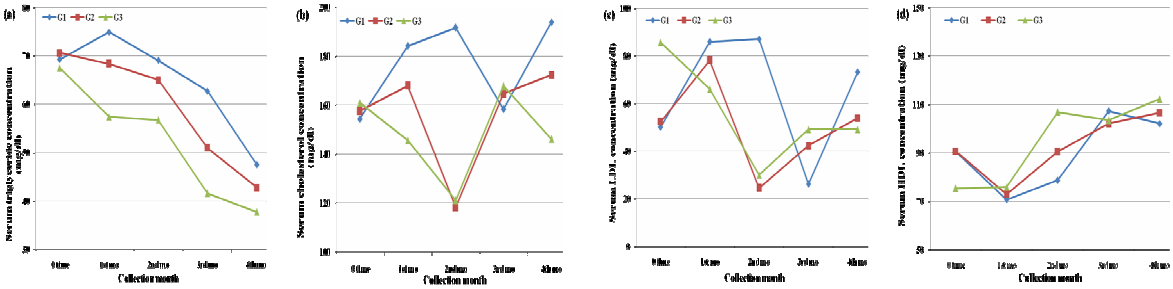

**Fig. 6. Change in concentration of triglycerides (a), total cholesterol (b), LDL (c) and HDL (d) in blood serum of buffalo bulls of different experimental groups at various collection months.**

It is worthy noting that the observed reduction in TG and TC in blood was associated with other reduction in their profile in seminal plasma. Generally, Astuti *et al*. (2011) reported that feeding animal on MO had certain amount of saponin led to good effect on health as expressed in low serum cholesterol and normal essential fatty acids concentration. Similar decrease was observed in rats (Nikkon *et al*., 2003; Lewis and Rader 2005; Pratik *et al*., 2013), who showed that, MOL had components can control in a mechanisms to involved in lipids elimination from the body. In addition, Lewis and Rader (2005) reported significant decrease in lipid profiles in rate fed MOL. In human treated with MOL, Seriki *et al*. (2015) found insignificant increase in blood serum HDL.

**Blood protein metabolites and transaminases activity:**

Data in Table 8 showed that serum urea concentration significantly (P<0.05) decreased only in G3, while concentration of uric acid and creatinine as well as AST and ALT activity significantly (P<0.05) decreased in both G2 and G3 as compared to G1. As affected by collection month, urea and uric acid concentrations significantly (P<0.05) decreased only during one month before semen collection and at the 3$^{rd}$ collection month, respectively. However, creatinine concentration and AST and ALT activity showed significantly (P<0.05) gradual reduction by advancing collection month. Effect of interaction between MOL treatment and collection month on all previous criteria was significant, reflecting different trend of change in the experimental groups at different collection months. Urea concentration was lower in G1 and G3 than in G2 at the 4$^{th}$ month (Fig. 7 a), uric acid concentration was lower in G2 than in G1 and G3 at the 1$^{st}$ month (Fig. 7 b), creatinine concentration was the lowest in G2, moderate in G3 and the highest in G1 at the 2$^{nd}$ month (Fig. 7 c), while AST activity was lower in G2 than in G1 and G3 at the 2$^{nd}$ and 3$^{rd}$ month (Fig. 7 d) of collection. These findings may suggest depended effect of MOL level at different collection months.

This indicated that MOL treatment had impact on decreasing protein metabolites and transaminase activity as a result of higher protein utilization MOL bulls than in control bulls. Similarly, Khalel *et al*. (2014) found that feeding lactating cows on MO up to 40% of the whole daily ration did not badly affects liver or kidney functions. Eshak and Osman (2013) observed that MO leave aqueous extract had a therapeutic action through enhancing of liver enzyme activities (AST and ALT) in irradiated rats by gamma irradiation. Also, Hoffmann *et al*. (2003) found high utilization of MO nitrogen making them available in the small intestine in an intact form led to lower blood urea level. On the other hand, Ahemen *et al*. (2013) reported insignificant effect of MOL meal diet on concentration of creatinine and urea as well as AST and ALT activity in blood serum of rabbit.

**Table 8. Mean and standard error of blood biochemical constituents of Egyptian buffalo bulls as affected by moringa treatment and sampling time.**

| Item | Urea (mg/dl) | Uric acid (mg/dl) | Creatinine mg/dl) | AST (U/L) | ALT (U/L) |
|---|---|---|---|---|---|
| Effect of treatment (T): | | | | | |
| G1 (control) | 15.90±0.95$^{a}$ | 1.49±0.07$^{a}$ | 0.87±0.03$^{a}$ | 64.06±2.17$^{a}$ | 22.15±0.67$^{a}$ |
| G2 (120 g/d/h MOL) | 14.66±0.76$^{a}$ | 1.25±0.06$^{b}$ | 0.71±0.02$^{b}$ | 55.20±1.91$^{b}$ | 18.27±0.82$^{b}$ |
| G3 (240 g/d/h MOL) | 12.09±0.88$^{b}$ | 1.16±0.09$^{b}$ | 0.66±0.05$^{b}$ | 54.28±2.64$^{b}$ | 17.24±0.77$^{b}$ |
| Effect of collection month (M): | | | | | |
| 0 time | 13.89±0.52$^{b}$ | 1.46±0.06$^{a}$ | 0.85±0.01$^{a}$ | 72.07±1.04$^{a}$ | 24.38±1.09$^{a}$ |
| 1$^{st}$ collection month | 18.62±1.24$^{a}$ | 1.50±0.08$^{a}$ | 0.81±0.06$^{ab}$ | 62.00±2.50$^{b}$ | 20.00±0.53$^{b}$ |
| 2$^{nd}$ collection month | 12.77±1.33$^{b}$ | 1.62±0.12$^{a}$ | 0.72±0.05$^{bc}$ | 61.79±2.98$^{b}$ | 17.98±1.02$^{c}$ |
| 3$^{rd}$ collection month | 12.69±1.25$^{b}$ | 0.86±0.07$^{b}$ | 0.69±0.07$^{bc}$ | 47.45±1.23$^{c}$ | 16.07±0.72$^{c}$ |
| 4$^{th}$ collection month | 13.12±0.70$^{b}$ | 1.06±0.09$^{b}$ | 0.68±0.04$^{c}$ | 45.91±1.73$^{c}$ | 17.67±0.87$^{c}$ |
| Effect of interaction (T x M): | | | | | |
| P-value | 0.000*** | 0.003** | 0.038* | 0.000*** | 0.221 |

**Means denoted within the same column with different superscripts are significantly different at P<0.05. 0 time : Pre-treatment and semen collection**

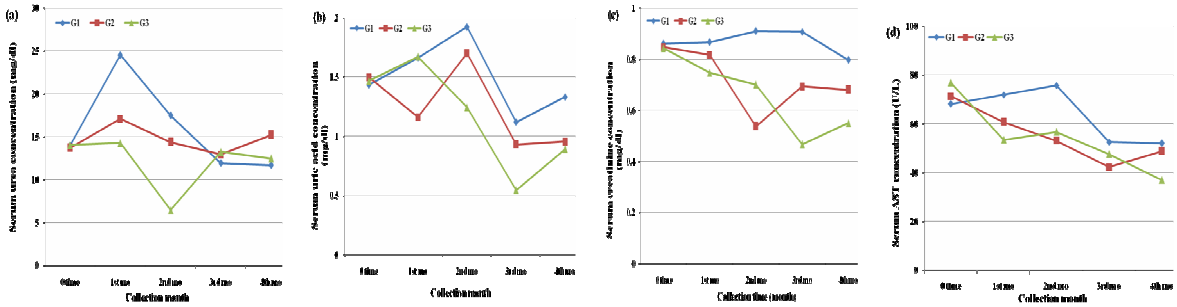

**Fig. 7. Change in concentration of urea (a), uric acid (b), creatinine (c) and AST (d) in blood serum of buffalo bulls of different experimental groups at various collection months.**

**Blood oxidative status:**

Results concerning blood serum antioxidant enzyme activity revealed that catalase, glutathione (GSH) and superoxide dismutase (SOD) significantly (P<0.05) increased in treatment groups treated with MOL, being the highest in G3. As affected by collection month contents of all antioxidant enzymes significantly (P<0.05) increased by advancing treatment and collection month, being at higher rate for SOD, followed by GSH and the lowest for catalase during month pre-treatment. The effect of interaction between MOL treatment and collection month on all antioxidant enzyme studied was significant (Table 9). These effects were reflected that MOL treatment interacted with sampling time, whereas catalase and GSH contents were positively affected by high level of MOL only at the last collection (Fig. 8 a and b). On the other hand, SOD content was positively affected by high level of MOL up to the 3rd collection month, and by low MOL at the last collection month (Fig. 8 c). The observed trends of change in oxidative enzymes may suggest that MOL supplementation had positive effect on increasing GSH contents during heat stress, being more than that on improving catalase and SOD contents. These findings indicated beneficial effects of MOL supplementation on antioxidant defense system during heat stress conditions.

**Table 9. Mean and standard error of blood oxidative enzymes of Egyptian buffalo bulls as affected by moringa treatment and sampling time.**

| Item | Blood oxidative status | | |
|---|---|---|---|
| | Catalase (mg/dl) | Glutathione (mg/dl) | Super oxidedismutase (mg/dl) |
| Effect of treatment (T): | | | |
| G1 (control) | 8.68±0.33$^{b}$ | 11.90±0.36$^{c}$ | 17.33±0.68$^{b}$ |
| G2 (120 g/d/h MOL) | 10.43±0.48$^{a}$ | 14.49±0.46$^{b}$ | 19.60±0.88$^{a}$ |
| G3 (240 g/d/h MOL) | 10.81±0.54$^{a}$ | 15.45±0.62$^{a}$ | 20.12±0.78$^{a}$ |
| Effect of collection month (M): | | | |
| 0 time | 7.85±0.16$^{c}$ | 10.34±0.13$^{d}$ | 13.33±0.36$^{e}$ |
| 1$^{st}$ collection month | 8.27±0.38$^{c}$ | 12.77±0.50$^{c}$ | 17.40±0.77$^{d}$ |
| 2$^{nd}$ collection month | 9.74±0.32$^{b}$ | 14.58±0.60$^{b}$ | 19.47±0.52$^{c}$ |
| 3$^{rd}$ collection month | 10.25±0.17$^{b}$ | 15.38±0.61$^{b}$ | 21.33±0.98$^{b}$ |
| 4$^{th}$ collection month | 13.76±0.68$^{a}$ | 16.68±0.53$^{a}$ | 23.54±0.41$^{a}$ |
| Effect of interaction (T x M): | | | |
| P-value | 0.000*** | 0.000*** | 0.003** |

**Means denoted within the same column with different superscripts are significantly different at P<0.05.**
***SOD: Supper oxide dimatease. 0 time: Pre-treatment and semen collection.**

Similar results were reported in rabbits treated with MO extract (El-Harairy *et al*., 2016) and in goats fed MOL by Babikera *et al*. (2017), who found high catalase content in the serum may be due to the high antioxidant activity of bio-constituents like saponin, alkaloid and flavonoid present in MOL. It is well known that antioxidant enzymes such as catalase, GSH and SOD are the main defense against free radicals which cause oxidative damage in animal organs. According to the present results, activity of antioxidant enzymes were markedly increased in blood of bulls in G2 and G3 as affected by MOL, which have a high antioxidant activity that can provide a health benefit to animals (Mbikay, 2012).

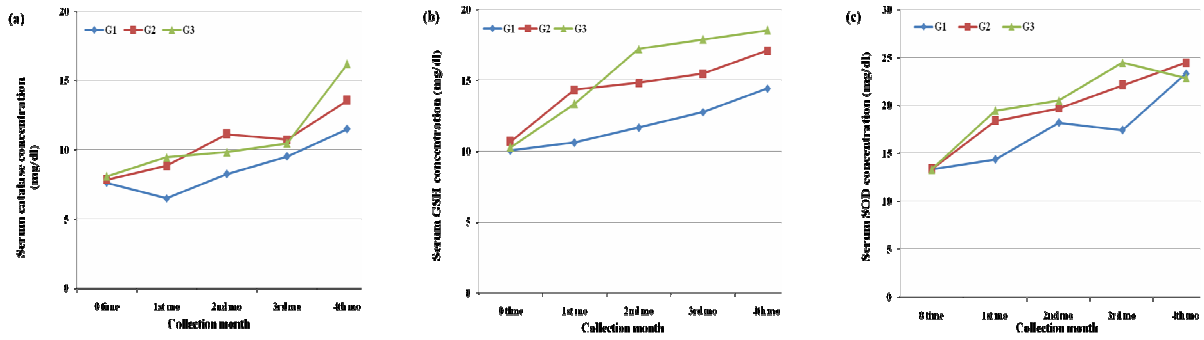


**Fig. 8. Change in content of catalse (a), GSH (b) and SOD (c) in blood serum of buffalo bulls of different experimental groups at various collection months.**

## CONCLUSION

The current study can conclude that, moringa oleifera leaves could be used as feed additive to help farmers for sustainable development of breeding bulls. Results of this study recommended that daily adding 240 g moringa oleifera leaves per buffalo bull for one month pre-semen collection or at a level of 8% of concentrate feed mixture in diets of buffalo bulls can improve quality and production of semen without any adverse effects on health status under hot climatic conditions in Egypt.

## REFERENCES

Abdel-Gawad, Eman, I.; Taha, A. T. and Ayoub, M. A. (2000). Monthely and seasonal variations in seminal plasma constituents of Barki Damascus male goats and their crosses under subtropical conditions. Proc., 3rd All African Conf. Anim. Agric. 11th Conf. Egyptian Soc. Anim. Prod. 491.

Afolabi, A. O.; Aderoju, H. A. and Alagbonsi, I. A. (2013). Effects of methanolic extract of moringa oleifera leaves on semen and biochemical parameters in cryptorchid rats. Afr J Tradit Complement Altern Med., 10(5):230-235.

Ahemen, T; Adakole, H. Abu and Lois, K. (2013). Iorgilim Physiological responses of rabbits fed graded levels of Moringa oleifera leaf meal (MOLM): Some aspects of haematology and serum biochemistry. Archives of Applied Science Research, 5 (2):172-176.

Akinyemi., A., Fadiyimu, A., Julius., Alokan, S., Adebowale, N., Fajemisin, O. (2010). Digestibility, Nitrogen balance and haematological profile of West Africa dwarf sheep fed dietary levels of Moringa oleifera as supplement to panicum maximum. Journal of American Science, 6 (10):634-643.

Amini, A. and F. Kamkar (2005). The effects of gossypol on spermatogenesis in NMRI mice. Iranian J Sci Technol Trans. 29: 123-133.

Amman, R. P. and Hammerstedt, R. H. (1980). Validation of a system for computerized measurements of spermatozoa velocity and percentage of motile soerm. Biol. Repro. 23: 647 – 656 ( A. B. A. 1981 ) Vol. 99 (8): 4508.

Andrabi, S.M.H. (2009). Factors affecting the quality of cryopreserved buffalo (Bubalus bubalis) bull spermatozoa. Reprod. Dom. Anim., 44: 552-569.

Annison, E.F.; Lindsay, D.B. and Nolan, J.V. (2002). Digestion and Metabolism. In M. Freer & H. Dove (Eds.), Sheep Nutrition. CABI/CSIRO, Wallingford New York, pp 95–118.

Anwar, F.; Latif, S.; Asharaf, M. and Gilani, A. (2007). Moringa oleifera: A food plant with multiple medicinal uses, Phytother Res. 21: 17-25.

Asma, S.; Farooq, A.; Maleeha, M. and Ammara, F. (2005). Antioxidant activity of different solvent extracts of Moringa oleifera leaves under accelerated storage of sunflower oil. Asian Jour. of Plant Sci. 4(6): 630-635.

Astuti, D. A.; Baba, A. S. and Wibawan, I. W. (2011). Rumen fermentation, blood metabolites, and performance of sheep fed tropical browse plants. Media Peternakan, pp. 201-206.

Babeker, E. A. and Abdalbagi, Y. M. (2015). Effect of feeding different levels of moringa oleifera leaves on performance, haematological, biochemical and some physiological parameters of Sudan Nubian goats. Online Journal of Animal and Feed Research. Vol. 5, Issue 2: 50-61.

Babikera, E. E.; Juhaimia, F. A. L.; Ghafoora, K. and Abdoun, K. A. (2017). Comparative study on feeding value of Moringa leaves as a partial replacement for alfalfa hay in ewes and goats. Livestock Science 195: 21-26.

Bartles, H.; Bohmer, M. and Heirli, C. (1972): Clin. Chem., Acta 37:193.

Bergmeyer, H.U. (1983). U.V. Method of catalase assay. In "Methods of Enzymatic Analysis" Vol: 3, Weinheim. Deer field Beach, Florida, p. 273.

Bhakat, M.; Mohanty, T. K.; Raina, V. S.; Gupta, A. K.; Khan, H. M.; Mahapatra, R. K. and Sarkar, M. (2011). Effect of age and season on semen quality parameters in Sahiwal bulls. Trop. Anim. Health. Prod. 43:1161-1168.

Blom, E. (1983). Sperm Morphology with reference to bull infertility. Ludhiana, First All-India symposium on Animal Reproduction, pp.61-81.

Bull, R.C.; Everson, D.O.; Olson, D.P.; Kelly, K.W.; Curtis, S. and Tzou, G. (1991). Concentration of serum constituents in cold-stressed calves from heifers and inadequate protein and (or) energy. Journal of Animal Science, 69, 853–863.

Caraway, W. T. (1963). Standard methods of clinical chemistry. In: Seligson D (ed). Academic, New York, London, 4, 239.

Chauhan, N. S.; Sharma, V.; Dixit, V. K. and Thakur, M. (2014). A review on plants used for improvement of sexual performance and virility. BioMed. Res. Int. Article ID 868062, 19 pages.

Cheah, Y. and Yang, W. (2011). Functions of essential nutrition for high-quality spermatogenesis. Adv. Biosci. Biotechnol. 2:182-197.

Chumark, P.; Khunawat, P.; Sanvarinda, Y.; Phornchirasilp, S.; Morales, N. P.; Phivthongngam, L.; Ratanachamnong, P.; Srisawat, S. and Klai-upsorn, S. P. (2008). The in vitro and ex vivoantioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of Moringa oleifera Lam. leaves. Journal of ethnopharmacology, 116(3): 439-446.

Daader, A. H.; Merai, I. F. and Nasr, A. S. (1993). Transaminasic and fructolytic activities of buffalo bull semen in relation to some physiological attributes of spermatozoa. In prospects of buffalo production in the Mediterranean and the Middle East. Proceeding of the international symposium. Eap. Pubblication no. 62:198.

Dacheux, J. L.; J. L. Gatti and F. Dacheux (2003). Contribution of epididymal secretory proteins for spermatozoa maturation. Microscopy Research and Technique, 61: 7-17.

Dafaalla, M. M.; Hassan, A. W.; Idris, O. F.; Abdoun, S.; Modawe, G. A. and Kabbashi, A. S. (2016). Effect of ethanol extract of Moringa oleifera leaves on fertility hormone and sperm quality of Male albino rats. World J. Pharm. Res. 5: 1-11.

Dhami, A. J. and Kodagali, S. B. (1987). Correlation between biochemical and enzymatic constituents of semen of Surti buffalo bulls. Indian J. Anim. Sci., 57: 1283.

Doumas, B.; Wabson, W. and Biggs, H. (1971): Albumin standards and measurements of serum with bromocresol green. Clin. Chem.. Acta, 31: 87.

Duncan, D. B. (1955). Multiple Range and Multiple F. Test Biometrics. 11: 1-42.

Eggum, B. O. (1970). Blood urea measurement as a technique for assessing protein quality. Br. J. Nutrition. 24: 983-988.

Eid, Y.; T. Ebeid and H. Younis (2006). Vitamin E supplementation reduces dexamethasone–induced oxidative stress in chicken semen. Br. Poult. Sci. 47: 350–356.

Ekins, R. P. (1984). Free hormones in blood: Concept and measurement. J. Clin. Immunoassay, 7: 163-180.

El-Harairy, M. A.; Abdel-Khalek, A. E.; Khalil, W. A.; Khalifa, E. I.; El-Khateeb, A. Y. and Abdulrhmn, A. M. (2016). Effect of Aqueous Extracts of Moringa oleifera leaves or arctium lappa Roots on Lipid Peroxidation and Membrane Integrity of Ram Sperm Preserved at Cool Temperature. J. Anim. and Poultry Prod., Mansoura Univ., Vol.7 (12 ): 467- 473.

El-Sherbieny, M. A. S. (2004). Physiological study on farm animals. PhD. Thesis Faculty of Agriculture Mansoura University, Egypt.

Eshak, M. G. and Osman H. F. (2013). Role of Moringa oleifera Leaves on Biochemicaland Genetical Alterations in Irradiated Male Rats. Middle-East Journal of Scientific Research, 16 (10): 1303-1315.

Fayomi, A.; Ahmed, A.; Musa, U.; Salami-Shinaba, J.O.; Ogedegbe, S.A. and Akanni, K. (2014). Moringa multi-nutrient blocks: formulation, production, and feeding trial under a tropical environment. International Journal of Science, Vol. 3: 67–84.

Fitri, A., Toharmat, T.; Astuti, D. A. and Tamura, H. (2015). The potential use of secondary metabolites in Moringa oleifera as an antioxidant source. Med. Pet. 38:169-175.

Friedewald, W. T.; Levy, R. I. and Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracen-trifuge. Clin. Chem. 18:499–502

Garg, S. K.; Makkar, H. P. S.; Nagal, K. B.; Sharma, S. K.; Wadhwa, D. R.; Singh, B. (1992). Oak (Quercus incana) leaf poisoning in cattle. Vet. Human Toxicol, 34: 161-164.

Ghasi, S.; Nwobodo, E. and Ofili, J. O. (1999). Hypocholesterolemic effects of crude extract of leaf of moringa oleifera Lam in high-fat diet fed Wister rats. Journal of Ethnopharmacology, 69: 21-25.

Ghasi, S.; Nwobodo, E.; Faizi, S.; Siddiqui, B.S.; Saleem, R.; Saddiqui, S. and Aftab, K. (1994). Isolation and structure elucidation of new nitrile and mustard oil glycosides from Moringa oleifera and their effect on blood pressure. Journal of National Production, 57: 1256-1261.

Hackett, A. J. and Macpherson, J. W. (1965). Some staining procedures for spermatozoa. A review. Can. Vet. J. 5: 55.

Henry, J. B. (2001). Clinical Diagnosis and Management by Laboratory Methods. Twentieth Edition, Page 485.

Henry, R. J. (1964): Clinical Chemistry, Harper & Row Publishers, New York. p.181.

Hoffmann, E.M.; Muetzel, S. and Becker, K. (2003). Effect of Moringa oleifera seed extract on rumen fermentation in vitro. Arch. Anim. Nutr. 57: 65-81.

Isikwenu, J. O.; Udeh, I. and Ifie, I. (2012). Hematological response, performance and economic analysis of cockerel chicks fed enzymes supplemented brewer's dried grains groundut cake-based diet Pakistan J. Nutrition, 11: 541-546.

Kastelic, J. P. (2013). Male involvement in fertility and factors affecting semen quality in bulls. Animal Frontiers. 3: 20-25.

Khalel, M. S.; Shwerab, A. M.; Hassan, A. A.; Yacout, M. H.; El-Badawi, A. Y. and Mona S. Zaki. (2014). Nutritional evaluation of moringa oleifera fodder in comparison with trifolium alexandrinum (berseem) and impact of feeding on lactation performance of cows. Life Science Journal,11(10): 1040-1054.

Khalifa, Walaa H.; F.M. Ibrahim, Aida I. El Makawy; Hafiza A. Sharaf; W.B. Khalil and Nagwa A. Maghraby (2016). Safety and fertility enhancing role of moringa oleifera leaves aqueous extract in New Zealand Rabbit bucks. Int j pharm; 6(1): 156-168.

Khan, A.; Yasinzai, M. M. and Kakar, M. A. (2015). Biochemical analysis of bovine (Boslndicus) seminal plasma. International journal of Advanced Biological and Biomedical Research, 3(4): 361-369.

Kumar, R.; Mohanarao, J.; Arvind. and Atreja, S.K. (2011). Freeze–thaw induced genotoxicity in buffalo (Bubalus bubalis) spermatozoa in relation to total antioxidant status. Mol. Biol. Rep., 38:1499-1506.

Lemma. A. and Shemsu, T. (2015). Effect of age and breed on semen quality and breeding soundness evaluation of pre-service young bulls. J. Reprod. And Fertil. 6: 35-40.

Lewis, G. F. and Rader, D. J. (2005). New insights into the regulation of HDL metabolism and reverse cholesterol transport". Circulation Research, 96 (12): 1221-1232.

Luqman, S.; S. Srivastava; R. Kumar; A.K. Maurya and D. Chanda (2012). Experimental assessment of Moringa oleifera leaf and fruit for its antistress, antioxidant, and scavenging potential using in vitro and in vivo assays. Evidence-Based Complementary and Alternative Medicine, 1-12.

Madesh, M. and Balasubramanian, K. A. (1998). Micro-titer plate assay for superoxide dismutase using MTT reduction by superoxide. Indian J. Biochem. Biophys. 35(3):184-188.

Martin, G. B.; Blache, D.; Miller, D. W. and Vercoe, E. (2010). Interactions between nutrition and reproduction in the management of the mature male ruminant. Animal 4:1214–1226.

Mbikay, M. (2012). Therapeutic potential of Moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: a review. Front. Pharmacol. 24: 1-12.

Mc Gowan, M.W.; Artiss, J.D.; Strandoergh, D.R. and Zak, B. (1983): Aperoxidase-Coupled method for the colorimetric determination of serum triglycerides. Clin. Chem. 29: 538-542.

Melo, V.; Vargas, N.; Quirino, T. and Calvo, C. M. C. (2013). Moringa oleifera L. An underutilized tree with macronutrients for human health Emir. J. Food Agric. 25 (10): 785-789.

Mullick, D. N. (1960). Effect of humidity and exposure to sun on the puls rate, respiration rate, rectal temperature and haemoglobin level, in different sexes of cattle and buffaloes. J. Agric. Sci. (Camb), 54: 391-394.

Nikkon, .F; Saud, A.; Haque, M. E.; Aragianis, K. and Mosaddik, M. A. (2003). Isolation of Aglycone of Deoxy- Niazimicin from Moringa oleifera Moringa oleifera Lam. and cytotoxicity, Rev. Latinoamer. Quim. 31(1): 5-9.

Nouman, W.; Siddiqui, M. T.; Basra, S. M. A.; Farooq, H.; Zubair, M. and Gull, T. (2013). Biomass production and nutritional quality of Moringa oleifera as a field crop. Turk. J. Agric. For. 37: 410-419.

NRC, National Research Council (1988): Nutrient requirements of dairy cattle 6th Rev. Ed., National Academy Press, Washington, D.C.

Nuhu, F. (2010). Effect of moringa leaf meal (molm) on nutrient digestibility, growth, carcass and blood indices of weaner rabbits. Thesis M.Sc., Agric. (Cape coast).

Omran, Fayza I. (2008). Impact of Thermo-physiological reaction on growth performance of buffalo calves. Ph. D. Thesis, Fac. Agric., Cairo Univ., Giza, Egypt, P 142.

Omran, Fayza I.; Shafie, M. M.; Ashour, G. H.; Youssef, M. M. and Laila R. Hassan (2013). Response of buffalo calves exposed to first and second acute thermal shocks. Egypt. J. Agric. Res., 91 (3): 1113-1128.

Oyeyemi, M.O; S.G. Olukole and O. Esan (2008). Sperm morphological studies of West African Dwarf Bucks treated with pumpkin plant (Cucurbita pepo). Int. J. Morphol. 26:121–126.

Payne, W. J. A. (1990). Cattle and buffalo meat production in the tropic. Intermediate Tropical Agriculture Series. Longman Sci. and Tech.

Perumal, P.; Srivastava, S. K.; Ghosh, S. K. and Baruah, K. K. (2014). Computer-assisted sperm analysis of freezable and nonfreezable Mithun (Bos frontalis) semen. Journal of Animals: Article ID 675031.

Prabsattroo, T.; Wattanathorn, J.; Iamsaard, S.; Somsapt, P.; Sritragool, O.; Thukhummee, W. and Muchimapura, S. (2015). Moringa oleifera extract enhances sexual performance in stressed rats. J. Zhejiang Univ. Sci. B 16: 179-190.

Pratik, K. C.; Vinodini, N. A.; Ranjith, S.; Rakshatha, R. and Anwar, A.(2013). Effect of Moringa oleifera leaf extract on cadmium induced renal toxicity in adult Wistar Albino rats. International Journal of Advanced Research, 1 (5): 162-165.

Prins, H. K. and Loos, J. A. (1969). "Glutathione," In: J. G. Yunis, Ed., Biochemical Methods in Red Cell Genetics, Academic Press. 127-129.

Purdy, P.H.; S.A. Ericksson; R.E. Dodson; K.L. Sternes and D.L. Garner (2004). Effects of flavonoids, silibinin and catechin on the motility of extended cooled caprine sperm. Small Ruminant Research, 55: 239-243.

Rasul, Z.; Anzar, M.; Jalali, S. and Ahmad, N. (1999). Effect of buffering systems on post-thaw motion characteristics, plasma membrane integrity and acrosomes morphology of buffalo spermatozoa. Animal Reprod. Sci., 59: 31.

Reitman, A. and Frankel, S. (1957): Colorimetric method for the determination of serum glutamic-oxaloacetic and glutamic-pyruvate transaminase. Amer. J. Clin. Path. 28: 56.

Richmond, W. (1973): Clin. Chem.., 19: 1350.

Roy, B.; Baghel, R. P. S.; Mohanty, T. K. and Mondal, G. (2013). Zinc and male reproduction in domestic animals: A Review. Indian J. Anim. Nutr. 30: 339-350.

Sarwatt, S.; Milang'ha, M.; Lekule, F. and Madala, N. (2004). Moringa oleifera and cottonseed cake as supplements for smallholders dairy cows fed Napier grass. Livestock Research for Rural Development, 16: 6.

Seriki, S. A.; Omolaso, B.; Adegbite, O. A. and Audu, A. I. (2015). Effect of moringa oleifera on lipid profile, blood pressure and body mass index in human. European journal of pharmaceutical and medical research, 2(7): 94-99.

Soliva C, Kreuzer M, Foidl N, Foidl G, Machmüller A and Hess H (2005). Feeding value of whole and extracted Moringa oleifera leaves for ruminants and their effects on ruminal fermentation in vitro. Animal Feed Science and Technology, 118: 47-62.

SPSS (2013). SPSS user Guide: Statistics SPSS.

Sreelatha ,S. and Padma, P.R.( 2009). Antioxidant activity and total phenolic content of Moringa oleifera leaves in two stages of maturity. J. Plant Food Human Nutrition, 64: 303 – 311.

Syarifuddina, N. A.; Tolengb, A. L.; Rahardjab, D. P.; Ismartoyoc,I. and Yusufb, M. (2017). Improving Libido and Sperm Quality of Bali Bulls by Supplementation of Moringa oleifera Leaves. Media Peternakan, 40:88-93.

Thom, E. C. (1959). The discomfort index. Weather-wise 12:57-59.

Trinder, P. (1969). Determination of blood serum glucose. Ann. Clin. Biochem, 6:24.

West, J. W. (2003). Effects of heat-stress on production in dairy cattle. J. Dairy Sci., 86: 2131.

Wu, G.; Bazer, F. W.; Davis, T. A.; Kim, S. W.; Li, P.; Rhoads, J. M.; Satterfield, M. C.; Smith, S. B.; Spencer, T. E. and Yin, Y. (2009). Arginine metabolism and nutrition in growth, health, and disease. Amino Acids 37:153-168.

Yanagimachi, R. (1982). Requirements of extracellular calcium ions for various stages of fertilization and fertilization related phenomena in the hamster. Gamete Research. 5: 323-344.

Young, S. S.; Eskenazi, B.; Marchetti, F. M.; Block, G. and Wyrobek, A. J. (2008). The association of folate, zinc and antioxidant intake with sperm aneuploidy in healthy non-smoking men. Hum. Reprod. 23:1014-1022.

## العائد من الإضافة الغذائية لأوراق المورنجا على خصائص السائل المنوي ، إجهاد الأكسدة ، الاستجابة الفسيولوجية وقياسات الدم لطلائق الجاموس المجهدة حراريا

**وائل محمد وفا[1]، حمدى عبدالله النجار[1]، عبدالفضيل عبدالحفيظ جبر[1] و محمد محمود رزق[2]**

**[1] معهد بحوث الانتاج الحيواني – مركز البحوث الزراعية – الدقي – الجيزة – مصر.**

**[2] هيئة المواد النووية – القاهرة – مصر.**

أجريت هذة الدراسة لتقييم الإضافة الغذائية لأوراق المورنجا على جودة السائل المنوي , إجهاد الأكسدة , التنظيم الحراري والحالة الصحية لطلائق الجاموس المجهدة حراريا. تم إستخدام 18 طلوقة جاموسي ناضج جنسيا وقسمت إلى ثلاثة مجاميع بكل منها 6 طلوقة. تم تغذية طلائق المجموعة الأولى على عليقة تتكون من مخلوط علف مركز , دريس برسيم وقش الأرز (كنترول) وغذيت طلائق المجموعة الثانية والثالثة على نفس مخلوط العلف المركز مضافا إليه أوراق المورنجا بمستوى 4 و 8٪ من مخلوط العلف المركز على التوالي لمدة شهر قبل تجميع السائل المنوي وأربعة أشهر كفترة تجميع للسائل المنوي. تم تجميع السائل المنوي مرتين أسبوعيا وقيم من حيث النسبة المئوية للحركة الفردية والحيوانات المنوية الحية والشواذ وذات الأكروسوم غير السليم. تم قياس استجابة الحيوان المنوي لاختبار الضغط الأسموزي (النسبة المئوية للحيوانات المنوية ملتوية الذيل) على 50 مل أسموز/لتر لمدة 30 دقيقة. تم قياس درجة حرارة المستقيم والجلد ومعدل التنفس والنبض. تم سحب عينات الدم قبل المعاملة وخلال أشهر تجميع السائل المنوي الأول , الثاني , الثالث والرابع لتقدير تركيز الهيموجلوبين , حجم الخلايا المعباءة , عدد كريات الدم الحمراء والبيضاء. تم تقدير تركيز البروتين الكلي , الألبيومين , الجلوبيولين , الجلوكوز , الجليسريدات الثلاثية , الكوليستيرول الكلي , الليبوبروتينات عالية الكثافة , الليبوبروتينات منخفضة الكثافة , اليوريا , حمض اليوريك , الكرياتينين والتستوستيرون , أيضا تم تقدير النشاط الإنزيمي لكل من AST , ALT , SOD , الكاتاليز والجلوتاثيون في سيرم الدم. تم أيضا تقدير كل من الجليسريدات الثلاثية , الكوليستيرول الكلي , النشاط الإنزيمي لكل من AST , ALT , SOD , الكاتاليز والجلوتاثيون في بلازما السائل المنوي. أظهرت النتائج إنخفاض معنوي في درجة حرارة المستقيم والجلد ومعدل التنفس والنبض في المجموعة الثالثة عن المجموعة الثانية والكنترول. زادت معنويا كل من درجة حرارة المستقيم والجلد ومعدل التنفس والنبض حتى الشهر الثالث لتجميع السائل المنوي ثم إنخفضت في الشهر الرابع تماشيا مع قيم دليل الحرارة والرطوبة. تحسنت معنويا النسبة المئوية للحركة الفردية , الحيوانات المنوية الحية , الشواذ , الحيوانات المنوية ملتوية الذيل وذات الأكروسوم غير السليم في المجموعة الثانية والثالثة مقارنة بالكنترول , كانت الأفضل معنويا في المجموعة الثالثة. جميع القياسات السابقة تحسنت معنويا مع التقدم في أشهر تجميع السائل المنوي. انخفض معنويا كل من الكوليستيرول الكلي والجليسريدات الثلاثية في بلازما السائل المنوي في المجموعة الثالثة مقارنة بالكنترول والمجموعة الثانية. إنخفض معنويا النشاط الإنزيمي لكل من AST , ALT بينما زاد معنويا النشاط الإنزيمي للكاتاليز , الجلوتاثيون و SOD في بلازما السائل المنوي للمجموعة الثانية والثالثة مقارنة بالكنترول , بينما إنخفضت معنويا الجليسريدات الثلاثية مع التقدم في أشهر تجميع السائل المنوي في حين لم يتأثر الـ AST. كان تركيز هرمون التستوستيرون في السيرم أعلى معنويا في المجموعة الثانية والثالثة عن الكنترول وكان الأعلى في المجموعة الثالثة. كان كل من حجم الخلايا المعباءة , الهيموجلوبين وعدد كريات الدم الحمراء عالي معنويا في المجموعة الثانية والثالثة عن الكنترول وكان الأعلى في المجموعة الثالثة , في حين أظهر عدد كريات الدم البيضاء معدل معاكس معنويا. حدثت زيادة معنوية لكل من الهيموجلوبين , عدد كريات الدم الحمراء والبيضاء بعد شهر من المعاملة ثم زاد الهيموجلوبين وعدد كريات الدم الحمراء معنويا , بينما إنخفض معنويا حجم الخلايا المعباءة وعدد كريات الدم البيضاء في الشهر الرابع لتجميع السائل المنوي. بالمقارنة بالمجموعة الكنترول والمجموعة الثانية زاد معنويا تركيز البروتين الكلي , الألبيومين والجلوكوز في السيرم في المجموعة الثالثة , في حين لم يتأثر الجلوبيولين بالمعاملات. مع التقدم في أشهر تجميع السائل المنوي زاد معنويا بشكل تدريجي تركيز البروتين الكلي , الألبيومين والجلوبيولين. إنخفض معنويا تركيز الجليسريدات الثلاثية والكوليستيرول الكلي في المجموعة الثانية والثالثة , بينما زادت معنويا الليبوبروتينات عالية الكثافة في المجموعة الثالثة مقارنة بالكنترول. لم تتأثر الليبوبروتينات منخفضة الكثافة بالمعاملات. مع التقدم في أشهر تجميع السائل المنوي إنخفض معنويا تركيز الجليسريدات الثلاثية وزادت معنويا الليبوبروتينات عالية الكثافة. أظهر معنويا تركيز الكوليستيرول الكلي والليبوبروتينات منخفضة الكثافة تذبذب متغير الاتجاه خلال أشهر تجميع السائل المنوي. أنخفضت اليوريا في السيرم معنويا في المجموعة الثالثة , بينما إنخفض حمض اليوريك , الكيرياتينين , AST وALT في المجموعة الثانية والثالثة مقارنة بالكنترول. إنخفضت اليوريا وحمض اليوريك خلال الشهر الأول قبل تجميع السائل المنوي و في الشهر الثالث على التوالي. في حين إنخفض الكيرياتينين , AST وALT مع التقدم في أشهر تجميع السائل المنوي. زاد معنويا النشاط الإنزيمي للكاتاليز , الجلوتاثيون و SOD في المجموعة الثانية والثالثة , وكان الأعلى في المجموعة الثالثة. زادت الإنزيمات المضادة للأكسدة معنويا مع التقدم في أشهر تجميع السائل المنوي , وكان أعلى معدل في SOD , يليه الجلوتاثيون وأقل معدل كان للكاتاليز أثناء الشهر قبل المعاملة. يستخلص من هذة الدراسة أنه يمكن إستخدام أوراق المورنجا كإضافة غذائية لمساعدة المربين في التنمية المستدامة لطلائق التربية. توصي نتائج هذة الدراسة بأنه يمكن إضافة 240جم أوراق مورنجا يوميا لطلائق الجاموس لمدة شهر قبل تجميع السائل المنوي أو بمعدل 8٪ من مخلوط العلف المركز في عليقة طلائق الجاموس ليحدث تحسن في جودة وإنتاج السائل المنوي دون أي آثار سلبية على الحالة الصحية تحت الظروف المناخية الحارة في مصر.