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

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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 mMol/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 oleifera* leaves, physiological parameters, hematological parameters.

INTRODUCTION

Buffaloes played an important role in livestock production by providing the milk, meat, leather and work draft force (Andradi, 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 (Lenma 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 (ω-linoleic), vitamins (Vit. A, C, and E) and minerals (Zn and Se) (Cheah and Yang, 2011). In Bali bulls, Syarifuddin 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 is 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 (Onran, 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., 2014), 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 feed 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 -70°C 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:

\[
THI = 0.8 \times AT\ °C + \left[ \frac{RH}{100} \times (AT\ °C \ - \ 14.4) \right] + 46.4
\]

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.

**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 week 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

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

<table>
<thead>
<tr>
<th>Experimental period</th>
<th>AT (°C)</th>
<th>RH (%)</th>
<th>THI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 time (Pre-treatment and collection)</td>
<td>24.13±0.85</td>
<td>33.00±1.58</td>
<td>68.88±0.86</td>
</tr>
<tr>
<td>1st collection month</td>
<td>29.63±1.46</td>
<td>51.03±0.71</td>
<td>77.85±1.83</td>
</tr>
<tr>
<td>2nd collection month</td>
<td>31.38±1.20</td>
<td>47.40±1.93</td>
<td>79.55±1.53</td>
</tr>
<tr>
<td>3rd collection month</td>
<td>32.50±0.41</td>
<td>49.73±2.64</td>
<td>81.49±0.64</td>
</tr>
<tr>
<td>4th collection month</td>
<td>30.75±0.43</td>
<td>34.90±0.42</td>
<td>76.71±0.53</td>
</tr>
</tbody>
</table>
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 aspartate (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 ALT, AST, 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 rate (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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Rectal temperature (°C)</th>
<th>Skin temperature (°C)</th>
<th>Respiration rate (time/min)</th>
<th>Pulse rate (pulse/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of treatment (T):</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>G1 (control)</td>
<td>39.00±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.83±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64.30±2.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>47.30±1.36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G2 (120 g/d/h MOL)</td>
<td>38.76±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.48±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.47±1.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.23±1.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G3 (240 g/d/h MOL)</td>
<td>38.29±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.92±0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>57.00±1.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.33±0.94&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Effect of collection month (M):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 time</td>
<td>38.31±0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.21±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.89±1.84&lt;sup&gt;d&lt;/sup&gt;</td>
<td>41.7±1.30&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; collection month</td>
<td>38.55±0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.27±0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.39±1.90&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>42.72±1.21&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; collection month</td>
<td>38.82±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.48±0.15&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>65.33±2.25&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>46.06±1.29&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; collection month</td>
<td>39.33±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.80±0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>71.33±1.99&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>52.44±1.34&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; collection month</td>
<td>38.42±0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>36.30±0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>60.67±2.15&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>42.39±1.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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: pretreatment 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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Sperm characteristics (%)</th>
<th>Effect of treatment (T)</th>
<th>Effect of collection month (M)</th>
<th>Effect of interaction (T x M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (control)</td>
<td>Individual motility: 65.52±0.55a</td>
<td>Livability: 63.64±0.36a</td>
<td>Abnormality: 29.80±0.27a</td>
<td>Curled tail: 60.89±0.43a</td>
</tr>
<tr>
<td>G2 (120 g/d/h MOL)</td>
<td>71.82±0.69b</td>
<td>69.22±0.65b</td>
<td>22.80±0.51b</td>
<td>66.67±0.69b</td>
</tr>
<tr>
<td>G3 (240 g/d/h MOL)</td>
<td>74.53±0.80a</td>
<td>72.16±0.78a</td>
<td>19.95±0.68a</td>
<td>69.78±0.95a</td>
</tr>
</tbody>
</table>

1st collection month: 66.94±0.60d | 62.92±0.35d | 28.78±0.23a | 60.25±0.49d | 27.17±0.24a |

2nd collection month: 68.89±0.83c | 66.58±0.58c | 24.60±0.49b | 62.93±0.62c | 25.46±0.45b |

3rd collection month: 72.22±0.87b | 69.35±0.73b | 23.24±0.69c | 68.49±0.74b | 23.97±0.59c |

4th collection month: 74.44±1.03a | 74.50±0.88a | 20.13±1.04d | 71.43±1.13a | 23.44±0.83c |

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.

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 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. 2 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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Some biochemicals and enzyme activity in seminal plasma</th>
<th>Effect of treatment (T):</th>
<th>Effect of collection month (M):</th>
<th>Effect of interaction (T x M):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol (mg/dl)</td>
<td>Triglycerides (mg/dl)</td>
<td>ALT (U/L)</td>
<td>Catalase (mg/dl)</td>
</tr>
<tr>
<td>G1 (control)</td>
<td>101.55±2.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.32±1.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.99±0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.21±0.52&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>G2 (120 g/d h MOL)</td>
<td>94.74±3.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.66±1.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.23±0.63&lt;sup&gt;d&lt;/sup&gt;</td>
<td>14.21±0.73&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>G3 (240 g/d h MOL)</td>
<td>86.46±3.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.16±1.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.55±0.81&lt;sup&gt;d&lt;/sup&gt;</td>
<td>17.00±0.84&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means denoted within the same column with different superscripts are significantly different at P<0.05. 0 time: pre-treatment and semen collection. AST: Aspartate 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, Ghazi 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.

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. Aherem et al. (2013) reported that diet 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 AST was 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.
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 (Ghazi 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).](image)

**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 in 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 in 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. Generally, 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.**

<table>
<thead>
<tr>
<th>Item</th>
<th>Packed cell volume (%)</th>
<th>Hemoglobin (mg/dl)</th>
<th>Red blood cells (x10^6/mm³)</th>
<th>White blood cells (x10^6/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Effect of treatment (T):</td>
<td>7.05±0.13^c</td>
<td>7.80±0.15^a</td>
</tr>
<tr>
<td>G1 (control)</td>
<td>31.39±0.35^c</td>
<td>7.93±0.17^a</td>
<td>7.49±0.11^b</td>
<td>7.41±0.12^b</td>
</tr>
<tr>
<td>G2 (120 g/d/h MOL)</td>
<td>33.69±0.19^b</td>
<td>9.15±0.19^b</td>
<td>7.94±0.15^a</td>
<td>7.15±0.11^c</td>
</tr>
<tr>
<td>G3 (240 g/d/h MOL)</td>
<td>34.84±0.15^a</td>
<td>10.05±0.20^a</td>
<td>8.00±0.20^c</td>
<td>6.63±0.14^c</td>
</tr>
<tr>
<td>0 time</td>
<td>34.51±0.14^c</td>
<td>8.34±0.22^c</td>
<td>7.39±0.08^b</td>
<td>8.09±0.16^a</td>
</tr>
<tr>
<td>1st collection month</td>
<td>33.54±0.31^b</td>
<td>9.07±0.27^b</td>
<td>7.36±0.13^b</td>
<td>7.42±0.12^b</td>
</tr>
<tr>
<td>2nd collection month</td>
<td>32.42±0.42^a</td>
<td>8.94±0.37^ab</td>
<td>8.02±0.12^a</td>
<td>7.66±0.10^b</td>
</tr>
<tr>
<td>3rd collection month</td>
<td>33.24±0.59^b</td>
<td>8.68±0.18^c</td>
<td>7.93±0.21^a</td>
<td>7.44±0.15^b</td>
</tr>
<tr>
<td>4th collection month</td>
<td>32.81±0.57^a</td>
<td>10.18±0.32^a</td>
<td>8.00±0.20^c</td>
<td>7.80±0.15^a</td>
</tr>
<tr>
<td>P-value</td>
<td>0.000***</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Total proteins (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
<th>Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of treatment (T):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1 (control)</td>
<td>6.93±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.22±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.71±0.16</td>
<td>60.84±1.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G2 (120 g/d/h MOL)</td>
<td>7.25±0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.17±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.08±0.14</td>
<td>62.64±1.19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G3 (240 g/d/h MOL)</td>
<td>8.74±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.73±0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.01±0.15</td>
<td>65.89±1.54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Effect of collection month (M):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 time</td>
<td>6.53±0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.47±0.03&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.75±0.25&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>59.89±1.35&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; collection month</td>
<td>6.84±0.35&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>3.10±0.22&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.74±0.26&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>64.43±1.31&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; collection month</td>
<td>7.30±0.22&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.75±0.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.56±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.25±2.25&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; collection month</td>
<td>8.67±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.47±0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.20±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.01±1.85&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; collection month</td>
<td>8.85±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.73±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.11±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>63.03±1.56&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>P-value</td>
<td>0.002***</td>
<td>0.000***</td>
<td>0.036*</td>
<td>0.000***</td>
</tr>
</tbody>
</table>

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 d) 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.

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).

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.
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).

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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Triglycerides (mg/dl)</th>
<th>Total cholesterol (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>HDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 time</td>
<td>64.70±1.84</td>
<td>176.54±6.27</td>
<td>64.50±7.92</td>
<td>89.84±2.60</td>
</tr>
<tr>
<td>1st collection month</td>
<td>66.91±2.19</td>
<td>165.95±7.32</td>
<td>50.24±4.67</td>
<td>92.54±2.30</td>
</tr>
<tr>
<td>2nd collection month</td>
<td>63.62±1.41</td>
<td>143.73±10.45</td>
<td>55.98±4.61</td>
<td>94.74±3.19</td>
</tr>
<tr>
<td>3rd collection month</td>
<td>51.75±2.43</td>
<td>163.62±5.27</td>
<td>39.19±4.12</td>
<td>104.27±2.05</td>
</tr>
<tr>
<td>4th collection month</td>
<td>42.64±1.26</td>
<td>170.86±9.15</td>
<td>58.80±8.00</td>
<td>106.97±1.30</td>
</tr>
</tbody>
</table>

Effect of treatment (T):
- Effect of collection month (M):
  - 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

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 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 3rd 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 4th month (Fig. 7 a), uric acid concentration was lower in G2 than in G1 and G3 at the 1st month (Fig. 7 b), creatinine concentration was the lowest in G2, moderate in G3 and the highest in G1 at the 2nd month (Fig. 7 c), while AST activity was lower in G2 than in G1 and G3 at the 3rd and 4th 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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Urea (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (control)</td>
<td>15.90±0.95a</td>
<td>1.49±0.07b</td>
<td>0.87±0.03c</td>
<td>64.06±1.17d</td>
<td>22.15±0.67a</td>
</tr>
<tr>
<td>G2 (120 g/d/h MOL)</td>
<td>14.66±0.76a</td>
<td>1.25±0.06b</td>
<td>0.71±0.02b</td>
<td>55.20±1.91b</td>
<td>18.27±0.82b</td>
</tr>
<tr>
<td>G3 (240 g/d/h MOL)</td>
<td>12.09±0.88a</td>
<td>1.16±0.09b</td>
<td>0.66±0.05b</td>
<td>54.28±2.64b</td>
<td>17.24±0.77b</td>
</tr>
</tbody>
</table>

Effect of treatment (T):
- **a**: Means within the same column with different superscripts are significantly different at P<0.05.
- **b**: 0 time: Pre-treatment and semen collection.

Effect of collection month (M):
- 0 time: 13.89±0.52b, 1.46±0.06b, 0.85±0.01a, 72.07±1.04a, 24.38±1.09b
- 1st collection month: 18.62±1.24b, 1.50±0.08b, 0.81±0.06b, 62.00±2.50b, 20.00±0.53b
- 2nd collection month: 12.77±1.33b, 1.62±0.12c, 0.72±0.05ab, 61.79±2.98b, 17.98±1.02c
- 3rd collection month: 12.69±1.25b, 0.86±0.07ab, 0.69±0.07ab, 47.45±1.23b, 16.07±0.72ab
- 4th collection month: 13.12±0.70b, 1.06±0.09b, 0.68±0.04ab, 45.91±1.73b, 17.67±0.87ab

P-value: 0.000*** 0.003** 0.000*** 0.221

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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Catalase (mg/dl)</th>
<th>Glutathione (mg/dl)</th>
<th>Super oxdizedismutase (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (control)</td>
<td>8.68±0.33b</td>
<td>11.90±0.36c</td>
<td>17.33±0.68b</td>
</tr>
<tr>
<td>G2 (120 g/d/h MOL)</td>
<td>10.43±0.48a</td>
<td>14.49±0.46b</td>
<td>19.60±0.88b</td>
</tr>
<tr>
<td>G3 (240 g/d/h MOL)</td>
<td>10.81±0.54a</td>
<td>15.45±0.62b</td>
<td>20.12±0.78b</td>
</tr>
</tbody>
</table>

Effect of treatment (T):
- **a**: Means within the same column with different superscripts are significantly different at P<0.05.
- 0 time: Pre-treatment and semen collection.

Effect of collection month (M):
- 0 time: 7.85±0.16, 10.34±0.13c, 13.33±0.36c
- 1st collection month: 8.27±0.38c, 12.77±0.50c, 17.40±0.75c
- 2nd collection month: 9.74±0.32b, 12.58±0.60b, 19.47±0.52b
- 3rd collection month: 10.25±0.17b, 15.38±0.61a, 21.33±0.98b
- 4th collection month: 13.76±0.68a, 16.68±0.53b, 23.54±0.41a

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 catalase (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


العدد من الإضافية الغذائية لأوراق المورنجة على خصائص السائل المنوي. إجهاد الأكاسيد. الاستجابة الفسيولوجية

وقديات المذابين والمغذيات الموجهة حارباً

وائل محمد وأ. حمد عبد الله الطيار. أ. حدي صغير وفي. محمد محمد رزق.


2. الهيئة المولدية – القاهر – مصر.

أجريت هذه الدراسة لقياس الإضافية الغذائية لأوراق المورنجة على جودة السائل المنوي. إجهاد الأكاسيد. التنظيم الحراري. القدة الصحية لطاقتة. 

الجامعة الموجهة حارباً. تم استخدام 18 طائفة جامعي ناضجة وتم تقسيمها إلى ثلاثة عينات معمولة لكل منها طائفة. تم تقسيم طاقم المغذيات الموجهة حارباً على الأوراق المورنجة بنسبة 20% و 8% من تهمة الأوراق المورنجة. وحقق تقدم في تجربة تجريب النتائج. في تجربة التخليق المركزة على التوقف لم تحقق نتائج سائلة مني. ولم تجرد أوراق تجريب النتائج. في تجربة التخليق المركزة على التوقف لم تحقق نتائج سائلة مني. ولم تجرد أوراق


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L. Animal and Poultry Prod., Mansoura Univ., Vol. 9 (9), September, 2017


