Growth performance, biochemical parameters and antioxidant status of heat-stressed calves fed on a diet containing Moringa oleifera leaves powder

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ABSTRACT

The growth performance, biochemical parameters, antioxidant status and thyroid hormones of growing heat-stressed calves fed on a diet containing Moringa oleifera leaves powder (MOLP) as a natural antioxidant were assessed in this study. Fifteen calves aged eight months and weighing 130-136 kg were randomly distributed into three supplemented groups with five calves each. The control group (M0) fed concentrate mixture without any supplement and the supplemented groups M1 and M2 fed concentrate mixture with 0.3% and 3.0% MOLP supplementation, respectively. The results demonstrated that MOLP supplementation significantly improved (P≤0.01) the final body weight, total weight gain and average daily gain in both M1 and M2 groups recording the highest values in M2 group. MOLP supplementation considerably (P≤0.01) reduced serum total cholesterol, triglycerides, low-density lipoprotein and malondialdehyde concentrations recording lower values in M1 group, while improved (P≤0.01) serum total antioxidant capacity and catalase activity where the highest levels were recorded in M1 group. Conversely, serum concentrations of high-density lipoprotein, total protein, globulin, thyroxin and superoxide dismutase activity were non-significantly elevated in both supplemented groups. Serum glucose and triiodothyronine concentrations were considerably increased (P≤0.01) in M1 group however, serum albumin and urea concentrations were significantly increased (P≤ 0.01) only in M2 group. Serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase activities and creatinine concentration were significantly decreased (P≤ 0.01) in the supplemented groups however, glutathione peroxidase activity was significantly increased (P≤ 0.01) in both groups. Therefore, dietary MOLP supplementation can be used to improve growth, biochemical and antioxidant parameters in heat-stressed calves without any adverse effects.

1. INTRODUCTION

Global warming is a major threat to ruminant production causing a massive reduction in production and reproduction (Gaughan et al., 2009). Heat stress increases lipid oxidants due to the higher generation of free radicals, which enhances the formation of reactive oxygen species (ROS) inducing oxidative stress (Altan et al., 2003). Thus, it is necessary to use some feed additives that able to reduce such oxidative damage such as Moringa oleifera leaves (Al-Juhaimi et al., 2020). Moringa oleifera has attracted attention due to its comprehensive antioxidant, nutritional and medicinal qualities (Makkar and Becker, 1997). Moringa leaves are a rich source of natural antioxidants including flavonoids, phenolic acids, carotenoids, alkaloids, thiocarbanilates, glucosinolates and antioxidant minerals and vitamins specially vitamin C, E and β-carotene that could reduce the intensity of lipid peroxidation by removing the free radicals and activating the antioxidant enzymes (Mbijak, 2012). Also, the Moringa leaves are used as hypcholesterolemic, hepato-protective, hypotensive, hypoglycemic, diuretic, antiulcer, antispasmodic, anticancer and analgesic agents (Asare et al., 2012). Therefore, the current study aimed to evaluate the impacts of dietary MOLP supplementation, a natural source of antioxidants, on growth performance, blood biochemistrys and antioxidant status of heat-stressed calves during the Egyptian summer.

2. MATERIAL AND METHODS

2.1. Ethical approval

On a private farm in El Salehia district of the Sharkia Governorate, Egypt, during the summer months of July and August 2020, this study was carried out. Benha University, Faculty of Veterinary Medicine, Egypt's Committee of Animal Care and Welfare (BUFVTM 45-09-23) gave its approval to all procedures.

2.2. Climatic conditions

Ambient temperatures (AT) and relative humidities (RH) were recorded during day and night using a thermohygrometer and the mean values were calculated and illustrated in Table (1).

Table (1) Averages of temperature, relative humidity and temperature-humidity index (THI) through the period of the study.

<table>
<thead>
<tr>
<th>Periods</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient temperature (°C)</td>
<td>35</td>
<td>37</td>
<td>33</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Relative humidity (%)</td>
<td>36</td>
<td>60</td>
<td>41</td>
<td>55</td>
<td>48</td>
</tr>
<tr>
<td>THI values</td>
<td>81.8</td>
<td>89.5</td>
<td>80.4</td>
<td>85.7</td>
<td>84.4</td>
</tr>
</tbody>
</table>

*weeks

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The temperature humidity index (THI) was calculated and recorded in Table (1) according to Amundson et al. (2006) formula. The mean values of THI (THI= 84.4) indicated that the growing calves were reared under very severe heat stress conditions (THI value >78).

2.3. Animals, management and feeding

Fifteen clinically healthy crossbred calves of both sexes, aged eight months and weighing 130-136 kg were randomly distributed into three supplemented groups with five calves each. The calves were housed in an open shaded yard surrounded by fences and supplied with internal barriers to separate between the groups. The concentrate mixture was offered daily at 10.00 a.m., formulated from the locally available feed ingredients with calculated nutritional allowance according to (NRC, 2001) guidelines to cover all essential nutrient requirements of the growing calves, rice straw was offered ad libitum and clean and fresh drinking water was freely available. Before the start of the study, all calves were given an adaptation period of 10 days during which they were gradually introduced to the supplemented diet. The control group (M0) was fed concentrate mixture without any supplement and the supplemented groups M1 and M2 were fed concentrate mixture supplemented with 0.3% and 3.0% MOLP, respectively. The dried Moringa leaves were milled using a hammer mill (1 mm mesh) to produce MOLP, packed in sacks and stored in a well-ventilated storeroom to be ready for mixing with other feed ingredients.

2.4. Growth performance

The live body weights (BW) were measured at the beginning of the study to obtain their initial BW and then measured biweekly. The calves were weighed individually in the morning before feeding and watering. Total body weight gain and average daily weight gain (ADG) were calculated at the end of the study.

2.5. Blood sampling

Blood samples were collected individually from all calves in the morning biweekly before feeding from the jugular vein. Blood samples were centrifuged at 3000 rpm for 20 min to separate serum which was preserved at -20°C in clean sterilized tubes for biochemical and hormonal examination.

2.6. Blood biochemical parameters

The biochemical parameters including serum total protein, albumin, creatinine, blood urea nitrogen (BUN), triglycerides, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and glucose were analyzed colorimetrically by using commercial kits (Spinpro, S.A. / S.A.U Ctra. Santa. Coloma, Spain) as described by the manufacturer. Globulin was calculated as the difference between total protein and albumin. Serum concentrations of triiodothyronine (T3) and thyroxine (T4) were determined by using 125I-RIA and antibody-coated tubes kit (DIA source ImmunoAssays S.A. Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium).

2.7. Serum antioxidant profile

The total antioxidant capacity (TAC) was assayed in serum by colorimetric method using commercial kits (Diamond Diagnostic Company, Giza, Egypt). Serum malondialdehyde (MDA) concentration and the antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities were measured by using ELISA kit (Cusabio Biotech. Co., Ltd. China).

2.8. Statistical analysis

All data were analyzed by one-way analysis of variance (One-way ANOVA) with Tukey’s post hoc comparison test using SPSS version 20 statistical software. Significant differences between the means of different treatments were assigned significant at (P<0.05).

3. RESULTS

3.1. Growth performance

As compared to the control, the results in Table (2) showed that MOLP supplementation significantly increased (P<0.01) the final BW, total weight gain and ADG in both M1 (156.65 kg, 24.05 kg and 0.80 kg/dl, respectively) and M2 groups (166.58 kg, 34.38 kg and 1.15 kg/dl, respectively) recording the highest values in M2. The biochemical parameters are presented in Table (3). The results showed that MOLP supplementation significantly reduced (P≤ 0.01) serum triglycerides, total cholesterol and LDL concentrations where the lowest values were recorded in M1 (78.98, 193.36 and 129.19 mg/dl, respectively), while HDL, total protein, globulin and T4 concentrations were non-significantly increased in both M1 and M2. Glucose and T3 concentrations were significantly higher (P< 0.01) in M1 (188.14 mg/dl and 1.62 ng/dl, respectively), compared to control. The current results showed significant decrease (P≤ 0.01) in ALT, AST, ALP activities and creatinine concentration in both M1 (32.53 U/L, 35.25 U/L, 224.20 U/L and 1.30 mg/dl, respectively) and M2 (34.01 U/L, 31.43 U/L, 202.80 U/L and 1.27 mg/dl, respectively) compared to control.

<table>
<thead>
<tr>
<th>Item</th>
<th>M0</th>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (kg)</td>
<td>132.0±0.1* 1.14</td>
<td>133.0±0.1* 2.11</td>
<td>132.2±0.1* 7.11</td>
</tr>
<tr>
<td>Final body weight (kg)</td>
<td>151.38±1.19</td>
<td>155.65±1.24</td>
<td>156.5±1.24</td>
</tr>
<tr>
<td>Total weight gain (kg)</td>
<td>19.38±0.15</td>
<td>24.05±0.18</td>
<td>34.3±0.20</td>
</tr>
<tr>
<td>Daily weight gain (kg/dl)</td>
<td>0.64±0.01*</td>
<td>0.80±0.01*</td>
<td>1.15±0.01*</td>
</tr>
<tr>
<td>Means with different letters (a, b, c) in the same row are significantly different at (P&lt;0.05).</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

3.2. Blood biochemical parameters

The biochemical parameters are presented in Table (3). The results showed that MOLP supplementation significantly reduced (P≤ 0.01) serum triglycerides, total cholesterol and LDL concentrations where the lowest values were recorded in M1 (78.98, 193.36 and 129.19 mg/dl, respectively), while HDL, total protein, globulin and T4 concentrations were non-significantly increased in both M1 and M2. Glucose and T3 concentrations were significantly higher (P< 0.01) in M1 (188.14 mg/dl and 1.62 ng/dl, respectively), while serum albumin and BUN concentrations were significantly increased (P< 0.01) only in M2 (3.21 gd/l and 53.35 mg/dl, respectively). The current results showed significant decrease (P≤ 0.01) in ALT, AST, ALP activities and creatinine concentration in both M1 (32.53 U/L, 35.25 U/L, 224.20 U/L and 1.30 mg/dl, respectively) and M2 (34.01 U/L, 31.43 U/L, 202.80 U/L and 1.27 mg/dl, respectively) compared to control.

<table>
<thead>
<tr>
<th>Item</th>
<th>M0</th>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>114.42±4.19*</td>
<td>78.98±2.24*</td>
<td>92.5±20.5*</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>399.90±15.88*</td>
<td>193.36±2.96*</td>
<td>262.14±2.38*</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>42.98±1.19*</td>
<td>48.38±1.21*</td>
<td>49.5±20.85*</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>334.0±15.87*</td>
<td>193.36±2.96*</td>
<td>262.14±2.38*</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.12±0.05*</td>
<td>6.34±0.17*</td>
<td>6.46±0.07*</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.04±0.03*</td>
<td>3.01±0.07*</td>
<td>3.2±0.04*</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>3.08±0.03*</td>
<td>3.33±0.19*</td>
<td>3.2±0.06*</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>41.62±4.19*</td>
<td>38.87±1.57*</td>
<td>53.35±1.73*</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.76±0.03*</td>
<td>1.30±0.04*</td>
<td>1.27±0.04*</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>47.79±1.33*</td>
<td>32.53±0.79*</td>
<td>34.01±1.45*</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>42.62±4.19*</td>
<td>35.25±1.81*</td>
<td>31.43±0.57*</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>377.40±25.61*</td>
<td>224.20±16.12*</td>
<td>202.80±3.74*</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>153.19±3.75*</td>
<td>188.14±9.68*</td>
<td>143.91±3.14*</td>
</tr>
<tr>
<td>T3 (ng/dl)</td>
<td>1.51±0.02*</td>
<td>1.62±0.02*</td>
<td>1.56±0.03*</td>
</tr>
<tr>
<td>T4 (U/L)</td>
<td>69.83±4.71*</td>
<td>78.24±4.49*</td>
<td>75.45±0.86*</td>
</tr>
</tbody>
</table>

Means with different letters (a, b, c) in the same row are significantly different at (P<0.05).
3.3. Antioxidant profile

According to Table (4) results, there was a substantial (P≤ 0.01) rise in serum TAC and CAT activity with MOLP supplementation where the greatest levels were recorded in M1 (47.60 and 2.44 ng/ml, respectively). Serum MDA concentration was considerably decreased (P<0.01) in the supplemented groups and the lowest value was observed in M1 (72.80 nmol/ml). GSH-Px activity was significantly increased (P<0.01) in both M1 and M2 (136.60 and 130.60 ng/ml, respectively), while SOD activity was non-significantly improved by MOLP supplementation.

Table (4) Effect of dietary MOLP supplementation on antioxidant parameters of the heat-stressed calves.

<table>
<thead>
<tr>
<th>Items</th>
<th>Moringa supplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M0</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>171.20±8.78*</td>
</tr>
<tr>
<td>TAC (ng/ml)</td>
<td>26.01±1.14*</td>
</tr>
<tr>
<td>CAT (ng/ml)</td>
<td>1.60±0.6*</td>
</tr>
<tr>
<td>SOD (u/ml)</td>
<td>99.60±7.00*</td>
</tr>
<tr>
<td>GSH-Px (ng/ml)</td>
<td>96.00±4.30*</td>
</tr>
</tbody>
</table>

MDA= malondialdehyde, TAC= total antioxidant capacity, CAT= catalase, SOD= superoxide dismutase, GSH-Px = glutathione peroxidase, means with different letters (a, b, c) in the same row are significantly different at (P≤ 0.05).

4. DISCUSSION

The improvement in the final BW, total weight gain and ADG of the heat-stressed calves in both supplemented groups were in accordance with Abdel-Raheem and Hassan (2021); Kekana et al. (2021) and Anwar et al. (2024) who reported significant increases in the final BW and ADG with Moringa oleifera leaf meal (MLM) supplementation as 15% and 20% in growing buffalo calves, 8.33-16.66 g/100 kg BW in pre-weaned Holstein calves and as 10% in cattle heifers. Conversely, Sherasiya et al. (2022) reported non-significant influence on the BW and ADG of crossbred calves with feeding 5% MLM ration. This result could be attributed to the high protein content in Moringa oleifera leaves (MOL) due to their optimal composition of amino acids, high content of digestible protein and good quality rumen by-pass protein (Jiwuba et al., 2016), besides their ability to improve the microbial protein synthesis in the rumen (Soliva et al., 2005). Also, MOL have high nutrient profile and some digestion-promoting effects through the gradual improvement of the nutrients digestibility including OM, CP, CF and NFE (El-Badawi et al., 2023). Therefore, this result suggested that MOLP supplementation as 3.0% in calves' diet is an excellent nutritional source for enhancing growth performance even under heat stress conditions. The current study showed that different levels of MOLP supplementation significantly decreased (P< 0.01) serum triglyceride, total cholesterol and LDL concentrations recording lower values in M1. Although MOLP supplementation caused non-significant increase in HDL levels, it induced significant (P≤ 0.01) decrease in the levels of LDL which is directly related to coronary heart diseases as a major atherogenic lipoprotein and appears to be the main target of any lipid-lowering agent like MOL as indicated in these results (El-Gindy et al., 2017). These results were in agreement with Abdel-Raheem and Hassan (2021); El-Badawi et al. (2023) and Margret et al. (2023) who reported significant reduction in triglycerides and total cholesterol concentrations with MLM inclusion in the diets of buffalo calves, Egyptian lactating buffalos and Murrah buffalo heifers. Halaby et al. (2013) demonstrated that MOL contain β-sitosterol and phenolic compounds which were identified to have documented cholesterol-lowering effects through reducing cholesterol biosynthesis and absorption of dietary cholesterol. Also, MOL reduces the activity of HMG-CoA reductase which is a key enzyme in cholesterol biosynthesis (Jain et al., 2010). The presented results showed significant increase (P≤ 0.01) in serum albumin concentration in M2 which could be attributed to the high quality and quantity of dietary protein in MOL and its well utilization by the animal (Meel et al., 2018). This result was previously observed with dietary MLM supplementation in growing buffalo calves (Abdel-Raheem and Hassan, 2021); Sahiwal calves (Margret, 2021) and Egyptian lactating buffalos (El-Badawi et al., 2023). On the contrary, non-significant change in serum albumin concentration was observed with MOLP supplementation in crossbred calves (Sherasiya et al., 2023). The current increase (P≤ 0.01) in serum BUN concentration in M2 may resulted from the high protein content in MOL and its increased metabolism, absorption and utilization (Mandal, 2021). Also, this slight increase can be useful in urea recycling and its reuse as a nitrogen source for the synthesis of microbial protein in the rumen which is vital for the growth and maintenance of ruminants (Yusuf et al., 2018). This result was in accordance with Khalel et al. (2014); Zeng et al. (2018) and Mandal (2021) who reported significantly higher BUN concentrations in Friesian cows, Holstein dairy cows and Sahiwal calves with MOLP supplementation. However, this result disagreed with (El-Badawi et al., 2023) who reported that BUN concentration decreased significantly in Murrah buffalo heifers with dietary MOLP supplementation. Serum creatinine concentration was significantly (P≤ 0.01) decreased in both supplemented groups, which may be related to the high content of very powerful antioxidants in MOL such as phenolic compounds and ascorbic acid that have a scavenging effect on the free radicals (Mandal et al., 2014) resulting in a renoprotective effect without alteration in the proper functioning of the kidney of the heat-stressed calves (Chukwuonye et al., 2013). This result agreed with the findings of Kekana et al. (2019) and El-Badawi et al. (2023) with MOLP supplementation as 60 g/cow/day in Jersey cows and 50 g/head/day in Egyptian lactating buffalos. Conversely, non-significant difference in serum creatinine concentration was observed with MOLP supplementation in crossbred calves (Sherasiya et al., 2023). It is evident from the findings of this study that MOLP-supplemented groups had significantly (P≤ 0.01) lower serum ALT, AST and ALP activities than the control. The decreased levels of these enzymes reflected the normal or enhanced liver function and indicated the hepatoprotective effect of Moringa leaves. Also, such an effect suggests that these levels as 0.3% and 3.0% may be safe to be used for MOLP supplementation in growing heat-stressed calves without any adverse effects on the liver tissue. This result was supported by the findings of Wafa et al. (2017); Elaidy et al. (2017) and El-Badawi et al. (2023) with MOLP supplementation in Egyptian buffalo bulls, suckling buffalo calves and Egyptian lactating buffalos. In contrast, Margret et al. (2023) reported non-significant differences in serum ALT and AST levels with MOLP supplementation in Murrah buffalo heifers ration. Suleiman et al. (2017) reported that antioxidants found in MOL including phenolic acid and flavonoids, with hepatoprotective properties like quercetin, may work together to lower ROS levels that damage the liver cells resulting in protecting the membrane integrity of hepatocytes and preventing enzyme leakage into blood. Therefore, this result indicated the ability of MOL to improve the functional status of the liver cells and preserve the health and safety of liver tissues under heat stress conditions (El-Kashef, 2022). The current increase (P≤ 0.01) in serum glucose concentration may be due to that MOL help in carbohydrate absorption and increasing energy metabolites such as glucose which indicated its positive
effect in improving the energy status of the animal (Khalel et al., 2014). This result was in close agreement with Kekana et al. (2021); Abdel-Raheem and Hassan (2021) and Margret et al. (2023) who reported significantly higher glucose concentrations with MOL inclusion in the rations of pre-weaned Holstein calves, growing buffalos calves at 15% and 20% and Murrah buffalo heifers as 20%. Conversely, non-significant difference in serum glucose concentration was reported by Sherasayia et al. (2023) in crossbred calves. The presented increase in T3 concentration was in tune with the findings of Wankhede et al. (2022) and El-Kashef (2022) who noticed that feeding diet containing MOL induced the highest T3 and T4 concentrations compared to the control group under heat stress conditions. This elevation of T3 concentration may be due to the high content of antioxidants in MOL which can alleviate the adverse effect of heat stress and enhance the physiological responses. Tomar et al. (2020) stated that Moringa oleifera is able to stimulate thyroid function and increase T3 concentration by enhancing the conversion of T4 to T3, the principal source of T3 generation. Therefore, this result indicated better adaptation of the calves to chronic heat stress with MOLP supplementation (Naga Raja Kumar and Narendra Nath, 2018). It is well known that the antioxidant enzymes including CAT, GSH-Px and SOD are the main defense against ROS which cause oxidative damage in animal organs. So, the observed reduced (P≤ 0.01) SMDA concentration with increased (P≤ 0.01) STAC concentration, CAT and GSH-Px activities indicated the antioxidative effect of MOL and their suitability to reduce oxidative stress and its impacts on heat-stressed calves. These results agreed with Zhan et al. (2017) and Kekana et al. (2021) who observed increased STAC and reduced SMDA concentrations with MOL supplementation in the diets of dairy cows and pre-weaned Holstein calves indicating suppressed oxidative stress. Also, a similar improvement in the enzymatic antioxidant system was observed by Wafa et al. (2017); Kekana et al. (2021) and El-Badawi et al. (2023) who found improved SOD, CAT and GSH-Px activities and reduced SMDA concentration by MOL supplemented diets of the Egyptian buffalo bulls, pre-weaned Holstein calves and Egyptian lactating buffalos indicating the beneficial effects of MOL supplementation on the antioxidant defense system during heat stress conditions. Conversely, these results disagreed with Sherasayia et al. (2023) who reported non-significant effect of MOL on SOD and GSH-Px activities in crossbred calves. This decrease in SMDA concentration resulted from the synergistic antioxidant activities of phytoconstituents such as polyphenols, flavonoids, anthocyanin, glycosides, alkaloids, carotenoids, thiocarbazamates and antioxidant minerals and vitamins specially vitamin C, E and β-carotene in MOL (Mbikay, 2012). Also, the high CAT and GSH-Px activities may contribute to the reduction of MDA concentration by the decomposition of hydroperoxides (Babiker et al., 2016). Therefore, these results indicated that Moringa supplementation was effective in raising the antioxidant status of the growing calves and reducing stress during hot climatic conditions in Egypt.

5. CONCLUSIONS

Moringa oleifera leaves powder supplementation as 0.3% in the diet of the growing heat-stressed calves supplied nutrients and secondary metabolites which improved the growth performance, enzymatic antioxidant status, thyroid hormones and reduced lipid profile and oxidative stress without any adverse effects on the health status (liver and kidney functions).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data.

6. REFERENCES


